Educación Química

Reflexión

Charles-Antoine-Theodore Villiers: sugars and alkaloids

Charles-Antoine-Theodore Villiers: azúcares y alcaloides

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Resumen

Charles-Antoine-Théodore Villiers (1853-1925) fue un químico francés muy prolífico que descubrió que la fermentación del almidón con ***Bacillus amylobacter*** producía dextrinas (celulisinas) sin ir acompañadas de maltosa y glucosa, proceso claramente diferente al que resultaba de las diastasis. Su descubrimiento de las ciclodextrinas, sin saberlo, se convirtió en un importante aporte industrial. Villiers llevó a cabo un extenso trabajo sobre los alcaloides (ptomainas) generados por personas enfermas e identificó dos nuevos. Dentro del tema de la toxicología (forense) propuso un método mejorado para destruir materia orgánica. Villiers con Tanret demostraron que la materia dulce secretada por el maná de Persia contenía melezitosa, Villiers intentó sin éxito separar el componente activo del curare (curarina).

Palabras clave: alcaloides; celulisinas; ciclodextrina; curare; forense; melezitosis; ptomainas.

Abstract

Charles-Antoine-Théodore Villiers (1853-1925) was a very prolific French chemist who discovered that the fermentation of starch with ***Bacillus amylobacter*** yielded dextrins (cellulisines) without being accompanied by maltose and glucose, a process clearly different from that which resulted from diastases. His discovery of cyclodextrins, without knowing it, became an important industrial contribution. Villiers carried extensive work on the alkaloids (ptomains) generated by sick persons and identified two new ones. Within the subject of toxicology (forensics) he proposed an improved method or destroying organic matter. Villiers and Tanret, showed that sweet matter secreted by camelthorn contained melezitose. Villiers tried unsuccessfully to separate the active component of curare (curarine).

Keywords : alkaloids; cellulisines; cyclodextrin; curare; forensics; melezitose; ptomains.

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Life and career (Anonymous, 2024; Bougault, & Cattelain, 1933; Damiens, 1933)

Charles-Antoine-Théodore Villiers (-Moriamé) (1853-1925) (Figure 1), was born on January 6, 1854, in Carcassonne (southern France), were his father managed the communal school. In 1865, the family moved to Paris where Antoine completed his basic education at the lycées Louis-le-Grand, Charlemagne, and Condorcet. After obtaining his diplomas of bachelier és-lettres (1870) bachelier és- sciences (1871), and licencié és-sciences physiques (1873), he worked as préparateur at the École Pratique des Hautes Études, in the Collège de France, with Marcelin Berthelot (1827-1907), while pursuing studies of pharmacie at the École Supérieure de Pharmacie de Paris and doctorate at the Faculté des Sciences in Paris. In 1880 he was awarded his degree of Pharmacien de 1re Classe, after defending a thesis about the composition, properties, and derivatives of several sweet substances, among them, the manna produced by *Alhagi maurorum*, the leaves of the walnut tree, and many other vegetables, and a honey from Ethiopia (Villiers, 1880a). The same year he received his degree of docteur ès-sciences after presenting a thesis about the esterification of mineral salts, where he proved that the mechanism of the chemical equilibrium between alcohols and mineral was much more complex than those in standard situations (Villiers, 1880b). The obtention of the doctorate was the initial step on the way to a successful career. During this period of this life, he served as student delegate for the teaching of physical and natural sciences at the Lycée Condorcet (Lycée Fontane, between 1874 and 1883).

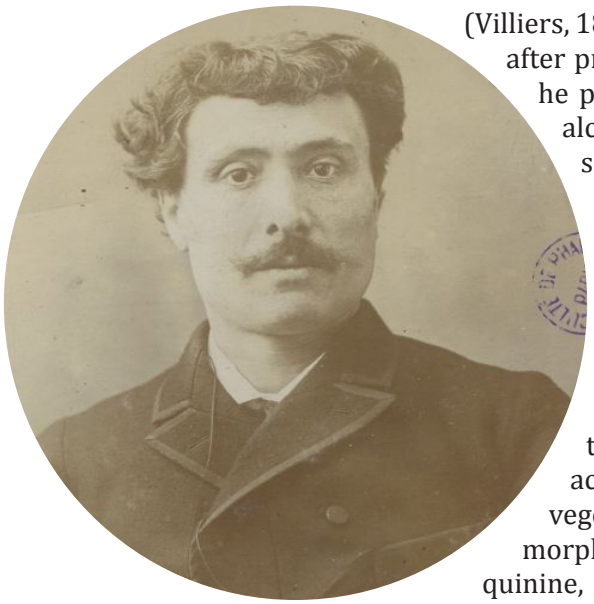

Figure 1. Charles-Antoine-Théodore Villiers (1853-1925).
Credit: Wikimedia, 2025.

In 1881 he won by competition, the position of Chef de Travaux Pratiques (head of practical work) in chemistry at the École Supérieure de Pharmacie and in 1882, the position of agrégé to the chair of toxicology and analysis, at the École (the door to an academic and research career), after presenting a dissertation about vegetable and animal poisons, among them the alkaloids (opium, morphine, codeine, thebaine, papaverine, narceine, meconin, laudanum, quinine, strychnine, nicotine, veratrin etc.), glucosides, volatile essences, resins, ptomaines, etc. (Villers, 1882). These studies led to his appointment to many important forensic commissions (i.e., Brouardel at al., 1893).

Between 1886 and 1895, Villiers oversaw the complementary course of analytical chemistry, a course that in 1895 became the pertinent chair. Villiers was appointed his first holder and kept this position for the next 30 years.

In 1894, Villiers was appointed Chevalier of the Légion d'Honneur.

Antoine Villiers passed away in Paris, on August 14, 1925.

Scientific contribution

Villiers wrote about 100 papers and books (i.e., Villiers, 1890, 1893; Villiers, Collin, & Fayole. 1909-1911, 1911) about his research activities in organic, inorganic, and analytical chemistry, sugars, alkaloids, etc. As customary for candidates to the Académie des Sciences,

he prepared a booklet describing his research activities and achievements (Villiers, 1885a). In addition to the few subjects presented below, Villiers found a new series of acid salts based on acetic acid (Villiers, 1877bc); discovered a method for characterizing sulfites in the presence of hyposulfites and sulfates (Villiers, 1887); determined the vapor pressure of mercury in rarefied gases and in air and its maximum value, at various temperatures (Villiers, 1913); with Bertault, discussed the methods used for determining the watering of milk (Villiers & Bertault, 1898); with Ernest Dumesnil he described a gravimetric method for determining ammonia in the state of ammonium chloride (Villiers & Dumesnil, 1900); with Marcelle Fayolle, he perfected a method for characterizing traces of chlorine and bromine in the presence or absence of iodine (Villiers & Fayole, 1894ab) and proved, that contrary to accepted opinion, ketones gave no coloration with the bisulfite solution of rosaniline (Schiff's reagent) (Villiers & Fayole, 1894c); etc.

Melezitoze

In 1833, Jean-François Bonastre (1783-1856) studied the origin of the sweet taste of the manna of Briançon produced by the young off shoots of larch (*Pinus larix*; mélèze in French) and concluded that it was due to the presence of a crystalline sugar, different from ordinary sugar. The sweet principle was found to be sparingly soluble in cold alcohol and a little more in boiling alcohol. The insoluble portion was seen to be formed a sort of network or small cells, which upon heating, swelled, giving first an odor of caramel, and then blackening and releasing abundant vapors. (Bonastre, 1833). Afterwards, Berthelot succeeded in extracting the sweet principle from the manna, with boiling alcohol and named it *mélézitose* (Berthelot, 1859). The cooled alcoholic extract deposited a mass composed of very small monoclinic crystals, which were substantially less sweet than ordinary sugar. Elemental analysis indicated a composition, by weight that corresponded to the formula of saccharides, $C_{12}H_{22}O_{11}$, accompanied by one molecule of water. The hydrate was efflorescent, melted at 140 °C, and lost its water easily. The rotatory power of the anhydride was $[a]_D$ = 94.1° (Berthelot, 1859).

In 1870, Berthelot received for examination, from a pharmacist in England, a sample of the manna exudated by camelthorn (*Alhaji Maurorum*), a small perennial highly branched thorny shrub of the Legume family, which was very abundant in Persia and was employed in folk medicine, as a purgative, diaphoretic, expectorant and diuretic, and food. Berthelot requested from Villiers to examine this material and try to identify its sweet principle (Villiers, 1877).

Villiers treated the manna with water, cleared the filtrate with animal charcoal, evaporated it to a syrupy consistency, and then left it alone. After some months, he found that the liquid had solidified into a collection of small, shiny crystals wetted by the mother liquor. The crystals were separated from the liquid by squeezing and vacuum pumping. The wrung crystals were dissolved in hot alcohol of 60° and left to cool. This resulted in the separation of voluminous white hydrated crystals, tasting less sweet than the manna. The crystals lost most their crystallization water by efflorescence at room temperature and became dehydrated completely by heating at about 100 °C in a vacuum over sulfuric acid. Elemental analysis indicated that they contained, by weight, 39.97% carbon, 6.99% hydrogen, and 53.04% of oxygen (by difference), corresponding to the formula of a hydrated sucrose $C_{24}H_{22}O_{22} + H_2O_2$ (Villiers is using the old values of atomic masses) (Villiers, 1877).

The formula of the anhydrous crystals, $C_{24}H_{22}O_{22}$, was confirmed by the percent weight lost during dehydration. These crystals melted slightly higher than 140 °C, had no action on Fehling's liquor, were dextrorotatory with rotatory power $[a]_D$ = +88.51°, and were oxidized by nitric acid to oxalic acid, without formation of mucic acid. Boiled with diluted sulfuric acid resulted in a slowly decrease of the rotatory power, which maintained its sign, until it reached that of glucose. The product of this reaction reduced Fehling's liquor. According to Villiers, all these characteristics indicated that the sweet component of the manna of Alhagi was identical with melezitose, the sugar described by Berthelot, present in the manna of Briançon (Villiers, 1877).

Villiers reported that the crystals of hydrated melezitose were clinorhombic prisms that showed only the *p* and *m* faces of the primitive form and the *g* faces. It contained also cane sugar and a syrupy material reducing Fehling's liquor. This cane sugar was the only component fermenting immediately with brewer's yeast (Villiers, 1877).

Inositol

Inositol was first isolated from muscles by Johann Joseph Scherer (1814-1869) and named inosite because of its sweet taste (Sherer, 1850)[1].

In 1877, Charles Tanret (1847-1917) and Villiers began a study of the sweet matter present in the leaves of walnut that culminated with the discovery of inositol in the vegetable kingdom (Tanret & Villiers, 1877, 1878, 1881).

Tanret and Villiers noted that the lixiviation of coarsely pulverized walnut leaves with lime milk and water led to the separation of very sweet substance, accompanied by oxidable materials and substances that reduced Fehling's liquid (Tanret & Villiers, 1877). The filtrate was treated with lead acetate, ammonia, diluted sulfuric acid, and baryte and the new filtrate evaporated in water bath to a syrupy consistency. Treatment of this product with a large amount of alcohol of 95% resulted in a viscous precipitate that was extracted with water and the resulting solution evaporated to a honeyed consistency and left to cool alone. The resulting crystals were separated and purified several times with alcohol of 50% and carbon black, yielding 3 grams of white hydrated crystalline material per kilo of dry leaves. In the air, these crystals effloresced and lost their water. This drying was very fast at 100 °C. Elemental analysis indicated that the dry material contained, by weight, 40.22% carbon, 6.52% hydrogen, and 53.26% oxygen (by difference), corresponding to the formula $C_{12}H_{12}O_{12}$. Tanret and Villiers remarked that this new body, which they proposed naming *nucite* (nucitol), had the same composition as inositol, although some properties were different (Tanret & Villiers, 1877).

Tanret and Villiers described nucite as a neutral substance, strongly sweet, crystallizing as clinorhombic crystals of relative density 1.54/10 °C, and melting at 208 °C without decomposition. It was very soluble in water, especially hot, insoluble in absolute alcohol, ether, and chloroform, and had no optical activity (sodium, length 20 cm, 0.25 g in 10 cm^3 of water). Nucite did not reduce Fehling's liquor, did not ferment with brewer's yeast, and did not react with diluted sulfuric acid. Oxidation with nitric acid did not produce nucic or oxalic acids (Tanret & Villiers, 1877).

[1] Scherer discovered that heating to dryness a mixture of inositol and a few drops of nitric acid and treating the residue with a drop of ammonia and a little of calcium chloride, generated a red color unique to inositol (except for quercitol). The derivatives of inositol also give colored solutions (Scherer, 1852). These results are known as the Scherer reaction.

Based on their results, Tanret and Villiers were unable to decide if their walnut leaf sugar was to be regarded as inositol, or only as an isomer of this body. They believed the best argument in favor or against was a comparison of the crystalline data of both substances (Tanret & Villiers, 1878). They mentioned that Paul Heinrich von Groth (1843-1927) had already done it for the sugars obtained from the leaves of ash (*Fraxinus excelsior*) and from walnut leaves and declared them identical. Both crystals had the same faces; the same planes bind cleavage, and the same angles (Groth, 1877). Tanret and Villiers repeated the procedure and compared the crystalline data of the sugar obtained from ash and from walnut leaves with those of the muscular inositol (obtained from horsemeat) and the sugar extracted from green beans, which they prepared for this purpose. The results were conclusive; all these sugars were the same material (Tanret & Villiers, 1878).

According to Tanret and Villiers, Wilhelm Marmé (1832-1897) had already shown that a sugar of the same composition was present in peas, green lentils, acacia, cabbage, digitalis, potato (the plant), asparagus, and in two cryptogams. They speculated that it was very probable that this sugar was also identical to inositol; the lack of the proper crystalline data did not allow verifying this assumption. Anyhow, the simultaneous presence of inositol in the plant and the animal kingdoms was an interesting fact (Tanret & Villiers, 1878).

The last publication of Tanret and Villiers was more detailed description of the results described previously, particularly regarding the action of Fehling's liquor, crystalline details, and new information about the action of nitric acid upon inositol (Tanret & Villiers, 1881).

Tanret and Villiers found that heating a solution of inositol with a small quantity of Fehling's liquor turned the liquor clear and green, but on cooling, it became blue again. Further heating produced a green precipitate. In the presence of an excess of inositol, the accompanying liquor was colorless but upon cooling in the air, the precipitate was partially dissolved, and the liquor recolored. Heating for a longer time, retained the remaining but the liquid turned into red oxide (Tanret & Villiers, 1881).

Dissolution of inositol in diluted nitric acid, followed by addition of sulfuric acid, precipitated the solute as crystallizable nitro-inositol. Heating the solution with concentrated nitric acid resulted a live reaction, with release of abundant nitrous vapors, but without formation of mucic acid or oxalic acid. Evaporation to dryness in a water bath left a white hygrometric residue, having a strong nitrous odor, and weighing as the original inositol used. This residue was very acid and was soluble in water, releasing nitrogen dioxide, nitrogen, and CO_2, and non-crystallizable. Heating the aqueous solution resulted in a brown liquid, which turned color upon cooling. Ammonia turned the residue strongly yellow, a color that was destroyed by organic acids. The residue reacted with metals producing a variety of colored salts. All the salts with calcium, barium, zinc, and mercury were intensely red. According to Tanret and Villiers, this result explained the reactions reported by Scherer, particularly the pink color produced with ammonia and a little of calcium chloride (Scherer, 1850, 1852; Tanret & Villiers, 1881).

Tanret and Villiers remarked that the inositol appeared in the walnut leaves from the beginning to the end of vegetation, while sucrose disappears during the leaf fall (Tanret & Villiers, 1881).

Curarine from *Strychnos nux-vomica*

The *strychnos* is a large genus of tropical trees and woody flowering vines belonging to the family *Loganiaceae* (order *Gentianellas*) and producing a berry that sometimes hold seeds extremely poisoning because of the alkaloids they contain.

In 1828, François Roulin (1796-1874) and Jean Baptiste Boussingault (1802-1887) carried some experiments on a sample of curare originating from Rio Negro (the largest tributary of the Amazon River) in the Orinoco area (Roulin & Boussingault, 1828). This was a black solid, and resinous material, extremely bitter, which upon heating swelled and hardly burned, without releasing any particular smell. It was easily soluble in water yielding an acid solution, which was not precipitated by ammonia, KOH, and alkali carbonates, and oxalates. It gave an abundant precipitate with the gallnut tincture, gallic acid, and gallates, which was completely soluble in alcohol and acids. According to Roulin and Boussingault, these results indicated that curare contained an alkaloid, probable strychnine. Additional chemical tests indicated that this assumption was wrong and that the active principle was another substance (curarine), soluble in water and alcohol, and insoluble in turpentine and ether. The aqueous solution neutralized acids and formed with HCl, sulfuric acid, and acetic acid, soluble salts, which seemed non-crystallizable. Roulin and Boussingault were unable to determine the chemical composition and formula of this principle (Roulin & Boussingault, 1828).

In 1865, William Thierry Preyer (1841-1897) reported that he had been able to extract the alkaloid present in the plant, by paying attention to the fact that the raw material contained foreign matter put in by the Indians to give the curare more consistency and keep it better (Preyer, 1865). Several different processes could do this purification. In one of them, the pulverized material was boiled with absolute alcohol containing a little of sodium carbonate, and the filtrate distilled to eliminate the solvent. The residue was dissolved in water, leaving the resinous matter behind. The curare in the aqueous extract was precipitated with an excess of mercuric chloride, filtrated, washed with water, and then suspended in a little water and decomposed by a stream of hydrogen sulfide. The active material was purified by repeating these steps several times. Preyer reported the preparation of curarine nitrate, acetate, hydrochloride and chloroplatinate. Analysis of the latter compound indicated that its formula was $C_{10}H_{15}N,PtCl_2$, indicating that curarine did not contain oxygen. Preyer described curarine as a hygroscopic substance, bitter, slightly acid, crystallizing as soluble salts in quadrilateral prisms, colorless, very soluble in water and alcohol, sparingly soluble in chloroform and amyl alcohol, insoluble in water, anhydrous ether, benzene, turpentine, and carbon disulfide. Concentrated pure sulfuric acid gave with curarine a persistent blue color, an effect that did not take place with strychnine; concentrated nitric acid produced a purple color, while potassium dichromate with sulfuric acid generated a purple color with both curarine and strychnine. According to Preyer, the reaction with sulfuric acid could be used to detect the presence of curarine in the liquids of animals that had been poisoned by this substance. For this purpose, it was enough to concentrate them by evaporation, extract the residue with absolute alcohol, evaporate it, and add a drop of sulfuric acid. The blue color signaled the presence of curarine (Preyer, 1865).

According to Gustave Planchon (1833-1900), the manufacture of curare was concentrated in four distinct regions of South America: (1) Upper Orinoco-Rio Negro, (2) Upper Amazon, (3) French Guiana, and (4) British Guiana. Planchon obtained samples of the roots, stems, and leaves of the important species of this region during the 1878 Paris International Exposition and provided botanical details about them (Planchon, 1880abc).

Berthelot asked Villiers to try to separate the toxic principle of the bark of the root of a strychnos used to prepare strong curare among the Piaora Indians inhabiting the Orinoco (Villiers. 1885b). As a previous step, Villiers treated the pulverized barks with ether to

remove the small amount of fat they seemed to contain, and then followed the extraction procedure described by Preyer. The residual material was treated with alcohol and the extract evaporated to eliminate the solvent. The new residue was exhausted with water and left a large quantity of resinous, insoluble matter, retaining the curarine in solution. The active principle was characterized by being insoluble in water and little soluble in neutral solvents, of which none was able to remove curarine from its aqueous solutions The principle was identified by the colored reaction it produced with concentrated sulfuric acid and with potassium bichromate and sulfuric acid, and by its physiological properties, identical as those with curare (Villiers, 1885b).

Villiers tried unsuccessfully to isolate pure curarine, using all known methods of extraction. He also tried to isolate the alkaloid by successive precipitations using various reagents, particularly the chlorides of mercury and platinum. The properties of these two precipitates suggested that they were not defined compounds (as claimed by Preyer), and that curarine was always accompanied by extractive substances precipitating at the same time as it under the action of the various reagents, so that the analysis of the precipitates formed by the various reagents led to different results. The fractional precipitation method gave similar results: the precipitates formed by curarine were amorphous, and its salts could not be crystallized (Villiers, 1885b).

The actual identification of curarine had to wait until 1935 when Harold King (1887-1956) reported the nature of tubocurarine, separated from a sample available in the Museum department of the Pharmaceutical Society of London (King, 1935). The several active principles were found to be amorphous quaternary alkaloids, accompanied, in the cases of tubocurare and pot curare, by inactive crystalline tertiary alkaloids. King isolated tubocurarine chloride and determined his composition, $C_{38}H_{34}O_6N_2Cl_2$, structure and properties (King, 1935).

Ptomains

In 1873 Francisco Selmi (1817-1881) reported that the putrefaction of dead bodies generated a small quantity of special alkaloids, cadaveric alkaloids, which the named *ptomains*. This name was because although most ptomains were volatile or very labile in contact with air, giving place to cadaveric odors, in contact with acid materials they developed very agreeable odors, such as orange flower and musk. The ptomains appeared as amorphous substances, oxygenated or non-oxygenated, non-volatile, or volatile, forming salts with acids, and in general behaving like vegetable alkaloids. In the free state, they exerted a toxic action upon the animal economy, such as slowing the heart action, loss of muscular contractibility, muscular flaccidness, etc. (Selmi, 1873, 1878).

This information, which was afterwards confirmed by many scientists, led Villiers to consider the possibility that ptomains were also the present in the organs of cholera patients (Villiers, 1885c). For these purposes, he was provided with the organs of two cholera patients that died in the Saint-Antoine Hospital, a few hours before (12 and 24 hours).

Villiers used Stas' method for isolating these particular alkaloids. This procedure included the following steps: (a) The tested matter, finely divided if necessary, was mixed with twice its weight of pure and very strong alcohol; (b) the liquid suspension was mixed with 0.5 to 2 grams of tartaric or oxalic acid and then heated in a flask to 60° or 75°C; (c) after complete cooling, the mixture was filtered, the insoluble residue washed with strong alcohol, and the filtered liquid evaporated under vacuum; (d) once the alcohol had

been eliminated, the residue was extracted with cold anhydrous alcohol, and the alcohol evaporated at room temperature under vacuum until almost dryness; (e) the acid residue was now dissolved in the smallest possible quantity of water and neutralized by adding powdered sodium or potassium bicarbonate in small portions until a fresh quantity produced no further effervescence of CO_2; and finally (f) the mixture was treated with four or five times its bulk of pure ether, left to settle, decanted, and left to dry in a very dry place (Stas, 1853).

Using this procedure, Villiers was able to separate about 0.02 g of the alkaloid hydrochloride from the intestine, and traces from the kidneys, liver, and heart blood. The last results seemed to indicate rapid elimination by the urine. The hydrochloride was neutral and crystallized in long and thin transparent needles, very deliquescent. The pure substance was a liquid having a pungent flavor and hawthorn odor. It was clearly basic and was set free by the alkalis and not by the alkaline bicarbonates. Villiers described many of its properties, for example, it produced colored precipitates with a variety of reagents: white with mercuric iodide, potassium iodide, tannin, and mercury dichloride; brown with iodide potassium iodide; yellow with brominated water and picric acid, yellowish white with gold chloride, violet with sulfuric acid, etc. (Villiers, 1885c).

Villers carried a few physiological experiments using an aqueous solution of the alkaloid. Small doses (1 mg to 2 mg), injected under the skin of a frog, did not produce a well-characterized action. The number of heartbeats of a frog was decreased a little at the beginning and dropped from to 34, then returned to 40. In another frog, the same dose resulted in muscular movements, which ceased quickly. A guinea pig was injected under the thigh with 6 mg of the hydrochloride dissolved in half a cubic centimeter of water. This led to periodic variations in the number of beats of the heart, and to a violent shacking of the forelimbs by a rapid tremor, which then invaded the hind limbs and quickly disappeared. The animal subsequently refused to take any food and death occurred only four days after the injection (Villiers, 1885).

The success in this subject led Villiers to investigate the existence of ptomains in other illnesses such as measles and diphtheria (Villiers, 1885d) and pathological urine (Villiers, 1885e).

Villiers used again Stas' method to remove an alkaloid from the lungs, kidneys, and liver of two children, aged 18 and 36 months, who had died of bronchopneumonia consecutive to measles. This substance induced fatty degeneration in the liver. The alkaloid was a volatile liquid having a pungent odor that excited sneezing. A drop of its solution placed on the tongue caused a long during caustic sensation and tingling. It was inert to tournesol and was set free by alkali bicarbonates. It gave amorphous colored precipitates with a variety of reagents, for example, white with mercuric iodide, mercuric chloride, and potassium iodide, yellowish white with brominated water, and gold trichloride, and brown with sulfuric acid. It did not react with platinum chloride, potassium dichromate, tannin, and picric acid. Villiers added that this new alkaloid was substantially different from the one withdrawn from the organs of cholera patients. It differed from it by its odor, its flavor, its weak alkalinity, the properties of its hydrochloride, by some of its reactions and by its physiological properties its mode of localization (Villiers, 1885d).

An identical alkaloid was removed from the lungs, kidneys of a two-year-old child who had died of diphtheria (Villiers. 1885d)

In 1876 Charles Bouchard (1837-1915) and Albert Cadier reported the existence of alkaloids in normal urine and their detection by means of a reagent based on the double iodide of potassium and mercury (Bouchard & Cadier, 1876). This result was seriously criticized by Villiers, claiming that these alkaloids existed only in pathological urine (Villiers, 1885e). Villiers carried several tests (including on himself) on the urine of healthy persons and people with several diseases. He looked for the presence of alkaloids using a general test for detecting their presence and not their nature, using a large volume of urine (1 to 2 liters), to be surer of their possible existence. He evaporated the urine to dryness and then extracted it with absolute alcohol. The filtered extracted was again evaporated to dryness and the residue treated with a drop of water. The resulting solution was treated with alkaline carbonates to displace the possible alkaloids, in the presence of ether. The ethereal solution was treated with a little of water acidulated with HCl to precipitate the alkaloids as their hydrochlorides. (Villiers, 1885e).

All the tests conducted on healthy persons gave a negative result (no alkaloid present). Villiers repeated the test on two occasions when he felt slightly indisposed, the first time by a slight bronchitis, and the second time by an ill-defined malaise, accompanied by fever. Here the results were different; they clearly indicated the presence of alkaloids in his urine. The same results were obtained with other ill persons. Villiers concluded that normal urine, discharged in good health, did not contain alkaloids, but that these could appear in even slight ailments, and probably in the case of more lesions, which went unnoticed because of habit. He speculated on the possibility that the alkaloids formed in some organs, passed through the circulation, and were eliminated by the kidneys. If elimination was slower than formation, true intoxication must occur. Ingestion of massive doses of water into the body could perhaps, facilitate the elimination of alkaloid materials. This was probably the real mode of action of most herbal teas administered in various diseases (Villiers, 1885e).

Fermentation

In 1891, Villiers began studying the fermentation of carbohydrates by means of the butyric ferment (*Bacillus amylobacter*) on potato starch. He chose the potato starch as initial material because this ferment converted it easily into dextrin (Villiers, 1891a). In the experimental procedure, a mixture of 50 g of potato flour, previously well diluted in water, to 1 liter of ordinary water. The flour was then converted into starch by a jet of vapor directed to the bottom of the flask, until the temperature increased to 100 °C. The mixture was then inoculated a few cubic centimeters of a culture of *Bacillus amylobacter,* and the flask sealed with sterilized cotton and left for several days in an oven 40 °C. The fermentation was left alone until the liquor no longer turned blue under the action of iodine. During this period, the shape of the bacillus changed from rectilinear, very mobile rods, to sticks thickened uniformly at their ends. All these steps were accompanied by the release of a very small amount of gas. The final liquor was slightly acid, smelling like butyric acid (Villiers, 1891a).

The main product of the reaction, dextrins, were accompanied by small amounts of secondary substances. The dextrins in the filtrate were precipitated with alcohol, purified by several washes with this solvent, and dried. The purified material appeared as a white, slightly sweet friable mass, very avid for water, with which they combined with release of heat. Repeated experiments indicated the formation of a variety of dextrins having rotational power between $[a]_D$ = +156° to +207.5°. These dextrins reduced the Fehling

liquor and were hardly converted into glucose by acidulated acids, even at 100 °C. Villiers found remarkable that the *bacillus amylobacter* converted starch into dextrin directly, without formation of maltose and glucose (Villiers, 1891a).

Villiers noticed that the action of *Bacillus amylobacter* upon potato flour produced, in addition to dextrins, about 3 g per 1000 flour, of a carbohydrate byproduct, which crystallized from the alcohol used for the precipitation of the dextrins (Villiers, 1891b). These crystals contained water and alcohol of crystallization and had a composition equivalent to the formula $C_{12}H_{10}O_{10}.C_4H_6O_2.10H_2O$. In contact with air, they become opaque, lost their alcohol, and absorbed water without changing their weight significantly. Villiers separated these crystals and recrystallized from hot water as shiny white crystals, barely sweet, unalterable in air, and having composition represented by the basic unit $C_{12}H_{10}O_{10} + 3H_2O$. The anhydrous crystals were very avid for water, like the dextrins, and in contact with water, they promptly recovered their original water of crystallization. Villiers named this new carbohydrate *cellulisine* and proceeded to determine its properties (Villiers, 1891b).

Cellulisine crystals were sparingly soluble in water, had rotatory power $[a]_D = +159.42^o$, they could be heated to high temperatures without melting, and at higher levels, it turned black and blistered. It did not ferment and did not react with Fehling's liquor and phenylhydrazine. Boiling diluted mineral acids converted it completely into glucose (Villiers, 1891b).

Villiers added that the total fermentation of potato starch left a residue of about 5% of the original weight, composed of a material having the composition of cellulose. He also found that other starches went through the same fermentation as potato starch, yielding at least another type of cellulisine (Villiers, 1891b).

Villiers repeated that he had shown that *Bacillus amylobacter* transformed starch into dextrin, without the latter being accompanied by fermentable products such as maltose and glucose (Villiers, 1891c). This transformation was clearly different from that which resulted from the action of the various diastases. The absence of maltose and glucose seemed to indicate a direct action of the organized ferment. He now repeated the experiment while measuring the rotatory power of the fermenting mixture and the pertinent change in color of the iodine tincture, and noticed that the value of $[a]_D$ first increased (10.30^o after half a day), reached a maximum (12.40^o after 3.5 days), and then decreased (10.32^o after 15 days), while the color of the tincture changed from dark blue to red, and finally to almost colorless. This data suggested that the ferment secreted a soluble product capable of performing the reaction, in the absence of any organized ferment. This product appears to form continuously, in very small proportions, and rapidly exhaust its action as it is developed (Villiers, 1891c).

Unfortunately, Villiers did not continue work on the subject, without knowing that his cellulisines were cyclodextrins, which would become an important commodity, sold in the thousands of tons.

Toxicology

In 1897, Villiers proposed a new and simpler method for destroying organic matter in toxicological examinations, based on using a manganese salt as oxidant (Villiers, 1897). The operation was conducted in a flask provided with a funnel shaped tube, which extended to the bottom, and by a tube ending in a vessel containing water, for absorption of the gases

released. The substance to be destroyed was introduced together with a dilute solution of pure HCl, followed by a few drops of a solution of a manganese salt and a small amount of nitric acid. The latter were added as needed. The mixture was heated to a moderate temperature, which was controlled according to the rate of gas evolution. The gases released consisted of almost pure CO_2 and nitrogen. According to Villiers, his apparatus and procedure yielded essentially the same results as the standard procedure with potassium chloride and HCl, but the operation was easier and safer. Organs, such as the liver, spleen, and lungs, were dissolved within minutes, while muscle fibers were first broken down and then dissolved after about an hour. The operation was completed as in the chlorate process (Villiers, 1897).

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