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Ariculo de Revisión

Léon-Albert Arnaud

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Abstract: Léon-Albert Arnaud (1853-1915) was a self-educated French chemist, who in spite of lacking formal academic education, became chair of chemistry at the Museum of National History in Paris. To him we owe the isolation of the poisons tariric acid and tanghinin, the first acetylenic compound discovered in vegetables; the discovery of the alkaloid cinchonamine, notable for the insolubility of its nitrate, the glucoside ouabain, sometime used in the treatment of cardiac affections, and the transformation of cupreine into quinine. He also showed the presence of carotene in the leaves of vegetables and determined the composition of digitalin.

Keywords: tariric acid (6-octadecynoic acid), carotene, natural rubber, cinchonamine, digitalin, ouabain, pyocyanine, quinines, tanghinin.

Resumen: León-Albert Arnaud (1853-1915) fue un químico francés auto educado que, a pesar de no tener educación académica formal, llegó a ser catedrático del Museo de Historia Natural de París. A él se le debe la separación de los venenos ácido tarírico y tanghinin, éste último el primer compuesto acetilénico descubierto en los vegetales; el descubrimiento del alcaloide cinchonamina, notable por la insolubilidad de su nitrato, el glucósido ouabain, que en su tiempo fue usado en la terapéutica del corazón, y la transformación de la cupreina en quinina. Asimismo, demostró la presencia del caroteno en las hojas de los vegetales y determinó la composición de la digitalina.

Palabras clave: ácido tarítico (ácido 6-ocatadecynoico), caroteno, caucho, cinchonamina, digitalin, ouabain, pyocyanin, quininas, tanghinin.

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Life and career [1,2,3]

Léon-Albert Arnaud was born on February 15, 1853, in Paris, the son of John-Elysée Arnaud, a watchmaker, and Julie Weber. In 1872, after completing his basic education at the Lycée Saint-Louis and École Monge, he began working as préparateur in the Muséum National d'Histoire Naturelle, under the chemist François Stanislaus Cloëz (1817-1883), an assistant of Michel Eugène Chevreul (1786-1889). In 1883, after the death of Cloëz, Arnaud was appointed head of the laboratory of Chevreul. In 1890, at the death of Chevreul, Arnaud tool over the chair of applied organic chemistry at the Museum, a position he kept until his death in 1915. Arnaud also served for 6 years as préparateur of the chemistry course at the École des Beaux-Arts and then as chemistry examiner for the special mathematics class at the Collège Chaptal (today Lycée Chaptal).

In 1887 the French Academy of Sciences awarded Arnaud one-half of the Jecker Prize on the basis of his discovery of the new alkaloid cinchonamine, his determination of the correct formula of carotene and the fact that it accompanied chlorophyll in the leaves of all plants, and the isolation of phytostearin, the vegetable cholesterol. The award committee was composed of Chevreul, Edmond Frémy (1814-1894), Auguste Cahours (1813-1891), Henri Debray (1827-1888), and Charles Friedel (1832-1899).[4] In 1888 the Academy presented Arnaud in second place [after Paul Schützenberger (1829-1897)] in the list of candidates to replace Debray in its Chemistry Section.[5]

Arnaud passed away in Paris, on March 27, 1915. At his request his body was incinerated in the Père-Lachaise cemetery.

Scientific contribution

Arnaud wrote about 45 papers on the subjects of biochemistry, biology, organic chemistry, vegetable immediate principles, etc. As customary to candidates the Académie des Sciences, he published a booklet describing his research and achievements.[6] In 1888 he lost to Paul Schützenberger (1829-1897) and never tried again.

Alkaloids

Cinchonamine

Arnaud began his first paper on the subject with a short review about the discovery of quinine and similar alkaloids.[7] In 1820 Pelletier and Caventou were the first to report the isolation of cinchonine and quinine in a pure state.[8] Afterwards, other chemists announced the finding of quinidine, cinchonidine, aricine, quinamine, and hydrocinchonine. Arnaud went on to announce the discovery of a new alkaloid, which he named *cinchonamine* because of its composition relation with cinchonine and quinamine. He found it in a red brown bark originating from the province of Santander in Colombia. Cinchonamine was present in this bark together with cinchonine. Arnaud treated the bark with limewater, dried the mixture, and then extracted it with boiling concentrated alcohol. The alcoholic extract was treated with excess of diluted HCl and the hydrochlorides of cinchonine and cinchonamine separated taking advantage that the later was little soluble in water at room temperature. The cinchonamine precipitated from water appeared as colorless and brilliant prisms, and as fine anhydrous needles when deposited from an alcoholic solution. In alcohol it was dextrorotatory with $\alpha_D = +122.2^o$ (alcohol of 97°, 22 °C), and turned blue litmus paper. It melted above 195 °C. Acids neutralized it forming salts that were little soluble. The chlorhydrate [$C_{12}H_{24}N_2O.HCl$] crystallized as prisms, which were little soluble in cold water and much less in aqueous HCl. The sulfate was very soluble in water and the nitrate was practically insoluble in nitric acid.[7] Arnaud also analyzed the barks of several varieties of Remijia (in particular Remijia purdiana and Remijia pedunculata) coming from the oriental areas of Colombia, and found they had some important differences: The bark of Remijia purdiana was very hard and heavier than water (relative density 1.128 to 1.180) and contained cinchonamine but not quinine, while that of Remijia pedunculata contained only quinine.[9]

In a following publication he described the extraction of cinchonamine and the preparation of several derivatives.[10] The pulverized bark was exhausted with water acidulated with sulfuric acid; the extract was filtrated and then boiled and precipitated with limewater. The calcareous precipitate was dried and then extracted with boiling ether under reflux. The ether dissolved the cinchonamine and left behind the resinous coloring impurities. The cold extract was washed with aqueous HCl and concentrated by boiling. The precipitated cinchonamine hydrochloride was purified by dissolution in boiling water and bleaching with animal carbon. The purified cinchonamine hydrochloride was treated with ammonia to eliminate the acid.

The resulting base was washed with water, dissolved in boiling ether, and left to crystallize by evaporation. Arnaud wrote that cinchonamine combined easily with acids, forming crystallizable salts, which were sparingly in water and very soluble in hot alcohol. He also described the preparation and properties of a large number of derivatives of the alkaloid, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, acetate, sulfate, tartrate, oxalate, malate, formiate, and citrate.[10] One important result was that cinchonamine nitrate was practically insoluble in water acidulated with any acid. This fact led Arnaud to develop an analytical method for detecting the presence of nitrates in a solution: It was enough to pour a few drops of cinchonamine hydrochloride in the solution; the presence of a nitrate resulted in the almost immediate precipitation of crystallized cinchonamine nitrate. This procedure permitted to detect as little as 1/1000 parts of potassium nitrate.[11,12] Arnaud and L. Padé demonstrated how this method could be used to detect the nitrates present in small quantities in vegetable tissues. For this purpose, it was enough to submerge a piece of the fresh tissue in an aqueous solution containing 1/250 parts of cinchonamine hydrochloride and a small amount of HCl. A microscopic examination of the tissue would show it was covered with crystals of cinchonamine nitrate.[13]

In 1889 and 1890 Arnaud published two extensive memoires describing in detail all the work he had done on cinchonamine, its preparation and properties, and the synthesis of its salts and derivatives.[14,15] The preparation of the alkaloid was somewhat different from the one described above: The pulverized bark was repeatedly extracted with a cold diluted solution of sulfuric acid; the alkaloids present in the resulting brown liquid were precipitated with ammonia. The precipitate was separated, washed with cold water, dried, and then extracted with boiling concentrated alcohol. After cooling, the alcoholic extract was treated with enough aqueous nitric acid to turn the solution frankly acid, and then left alone for a few days in a cold place. This resulted in the formation of an abundant precipitate of cinchonamine nitrate, little soluble in water. The separated crystals were purified by repeated recrystallization from boiling alcohol, in the presence of animal carbon. The nitrate was then decomposed by means of ammonia. [15]

Pure cinchonamine appeared as white and brilliant crystals, slightly bitter, little soluble in hot water and very soluble in cold and boiling alcohol, and in ether, chloroform, carbon disulfide, and benzene. Arnaud provided a detailed crystallographic description of the crystals. Cinchonamine had all the properties of an alkaloid: its alcoholic solution turned litmus blue, it combined easily with acid yielding crystallizing salts, its alcoholic solution was not colored by ferric chloride, by aqueous chlorine or ammonia, etc. An elemental analysis indicated that it contained, by weight, 77.17 % carbon, 8.22 % hydrogen, and 9.35 % nitrogen, corresponding to the formula $C_{12}H_{24}N_2O$, in between those of cinchonine, $C_{12}H_{22}N_2O$, and quinamine $C_{12}H_{24}N_2O_2$. In addition to the derivatives mentioned above, Arnaud provided details about the preparation and properties of the chloroplatinate and thiocyanate salts, the results of the reaction with methyl iodide, ethyl iodide, and potassium permanganate, as well as more examples of the use of cinchonamine nitrate for detecting the presence of nitrogen in plant tissues. [15]

Arnaud and Charrin wrote that cinchonamine sulfate was a very toxic compound; it usually provoked tonic-clonic seizures in the following doses of mg/kg, for sub-cutaneous injections containing 15 mg/cm^3 water: frogs 360; rabbit 61; dog 23; and man 17. [16]

Cupreine

Edouard Grimaux (1835-1900) and Arnaud carried a series of investigations related to quinine. In their first paper they exposed their procedure for converting cupreine, $C_{19}H_{22}N_2O_2$, a base extracted from the bark or seeds of *Quina cuprea* or *Remijia pedunculata*, into quinine, $C_{20}H_{24}N_2O_2$. [17] Inspection of the formulas showed that both compounds were linked in the same manner that phenol, C_6H_6O, was related to its methyl ether, C_7H_8O. The experimental results proved this assumption to be correct. Grimaux and Arnaud started from cupreine that was carefully purified to eliminate any possible traces of quinine. A mixture of this cupreine with sodium dissolved in methanol and methyl iodide was heated under reflux for several hours. This reaction resulted in the formation of quinine or methylcupreine, and a side reaction where the methyl iodide united with the nitrogen atom to form the iodomethylates of quinine. These byproducts were easily eliminated by crystallization in water and in alcohol: The separated quinine monoiodomethylate appeared as fine white needles and the di-iodomethylate as yellow prims.

Grimaux and Arnaud remarked that these two compounds were easily confounded with the two quinine iodomethylates because of the similarity of their fusion points. If the reaction was carried on in a closed vase, using methyl iodide mixed with a large excess cupreine and sodium in methanol, the only product was methylcupreine diiodomethylate having the same properties as the corresponding natural product, as shown by its melting point, rotatory product, iodine content, and its producing blue fluorescence when dissolved in sulfuric acid. Using methyl chloride instead of methyl iodide resulted in the formation of quinine in the free state, which was transformed into quinine sulfate by standard procedures. The sulfate appeared as light needles, grouped in stacks, having a strong bitter taste, and presenting blue fluorescence when dissolved in aqueous sulfuric acid. [17]

In the following paper Grimaux and Arnaud suggested that the derivatives of cupreine parallel to those of quinine [having the general formula ($C_{19}H_{21}N_2O,OR$), where R represented any group] be given the generic name *quinines.* [18,19] The first member of the series corresponded to quinethyline, $C_{21}H_{26}N_2O_2$. This base presented with quinine the gender homology that existed between ethyl phenoxide and methyl phenoxide. Grimaux and Arnaud indicated that quinethyline could be prepared by reacting at 120-130 °C ethyl chloride or bromide with a mixture of cupreine in methanol and sodium, or using instead, ethyl nitrate. The last procedure was preferable. The resulting quinethyline was an amorphous white solid composed of hydrates melting at 60 °C and becoming anhydrous at about 120 °C. The anhydrous form melted at 160 °C, was very soluble in ether, alcohol, and chloroform. It was levorotatory with α_D = -169.4° (in absolute alcohol). Grimaux and Arnaud reported also the preparation and properties of the acid and neutral sulfates of quinethyline. [18,19] In a following paper Grimaux and Arnaud informed that they had used the same procedure to prepare the propyl, isopropyl and amyl derivatives of cupreine. [20] Quinopropyline, $C_{19}H_{21}N_2O$, OC_3H_7, was prepared by reacting at 110 °C propyl nitrate and propanol with a mixture of cupreine in methanol and sodium, during 24 h. The resulting base appeared as a white powder hydrate melting above 100 °C. After desiccation under vacuum it melted at 164 °C without alteration. The isopropyl isomer was prepared by the same procedure. The purified product melted at 154 °C. Quinamyline was prepared by reacting at 105 °C amyl chloride with a mixture of cupreine in amyl alcohol and sodium, during 15 h. Grimaux and Arnaud reported the preparation of the sulfates of all the above compounds. [20]

A final paper on the subject repeated with more details the transformation of cupreine into quinine and homologous bases. [21]

Other natural products

Ouabain

Ouabain can be found in the roots, stems, leaves, and seeds of the *Acokanthera schimperi* and *Strophantus gratus* plants, native to eastern Africa. The Somali natives traditionally used the poisons derived from this genus of plants as an arrow poison, which was extracted from the branches and leaves of the plant by boiling them with water for 3 to 4 hours over fire. The resulting liquor was poured into a smaller pot, mixed with the juice of any Acacia, and the mixture stirred and concentrated by heating. The finished black tar-like juice, called *ouabe*, was then smeared on the tip of the arrow.

Arnaud and Alphonse Trémeau de Rochebrune (1836-1912), a physician and botanist, were charged by the French Ministry of Public Instruction to make a chemical and toxico- physiological study of this substance. They used for this purpose a large amount of the tree brought to them by George Revoil (1852-1894), the honorary consul of France in Somalia. [22] In a preliminary experiment, they found that the roots contained a glucoside precipitable by tannin. It was known that ouabain acted as a cardiac poison (producing a rapid systolic heart arrest). The poison was inactive only in extremely small doses. According to Rochebrune and Arnaud, a sub-cutaneous injection of 0.025 mg of crystalline ouabain stopped the heart of a frog after six minutes; and an injection of 0.012 5 mg did it in nine minutes. The experiments with higher animals indicated that the limiting toxic dose for a guinea pig or for a dog was 0.05 mg/kg of animal, with death occurring within 25 min. A rabbit resisted a slightly larger dose. [22]

Arnaud wrote that the Somalis prepared their arrow poison from the aqueous extract from the trunk and the roots of the tree named Ouabaio, which grew spontaneously on the slopes of Somalia Mountains. According to the botanists of the Museum, Adrian-René Franchet (1834-1900) and Jules Poisson (1833-1919), the tree belonged to the genus Carissa, of the Apocynaceae family, and was closely related to *Carissa schimeperi*, native from Abyssinia. [23]

Arnaud used water extraction to separate, a crystalline matter that seemed to have all the toxicity of the water extract. This substance provoked rapid death when injected subcutaneously or intravenously and was non-toxic when absorbed by the stomach. The Fehling liquor indicated that it contained a free reducing sugar, most probably glucose; with phenylhydrazine it produced an osazone derivative having the same melting point as phenyl glucosazone. An interesting result was that the bleached water extract was levorotatory, showing that the dextro rotatory power of glucose was compensated by the rotatory power of the active substance. [23] Arnaud's process was based on successive extraction with cold water of the tree cut in pieces. The resulting brown liquor was filtered and cleared of its coloring matter by means of lead acetate, followed by treatment with a stream of H_2S. It was then concentrated by boiling until it achieved a syrupy state, followed by addition of alcohol of 85° and further evaporation at a lower temperature. This resulted in the formation of a crystalline mass, which was separated by vacuum from the accompanying liquid. The crystals were purified by successive solution and recrystallization from alcohol of 85°. The yield of the complete process was only 3 g/kg of tree pieces. [23]

The purified material, which Arnaud named ouabain, appeared as very thin rectangular plates, pearly white, odorless, a little bitter, and having no action of litmus paper. They were little soluble in cold water (0.65g/100 g of water) and very soluble in boiling water; they were very soluble in cold alcohol, insoluble in ether, chloroform, and anhydrous alcohol, and precipitable by tannin. Ouabain formed a hydrate (containing 17.5 % weight of water) that lost most of its water at about 130 °C and reabsorbed rapidly upon cooling. The substance boiled neatly at 180 °C; diluted acids split it yielding a reducing sugar having rotatory power α_D = -34° (dissolved in hot water). Elementary analysis indicated that it contained, by weight, 59.93 % carbon, 7.62 % hydrogen, and 32.45 % oxygen, corresponding to the formula $C_{30}H_{46}O_{12}$.[23]

In a following paper Arnaud wrote that the natives of Gabon used an arrow poison named inée or onaye, extracted from the seeds of Strophantus hispidus (or Kombé), which also acted as a cardiac arrester. In 1871-1872 Ernest Hardy (1826-1890) and Narcisse Gallois (1831-1887) reported that these seeds contained a crystalline substance, strophantin, which seemed to be the active component of the poison.[24] Afterwards, Blondell Catillon determined some properties of strophantin, without indicating its composition.[25] Arnaud noted that the properties of strophantin and ouabain were very similar: both originated from a plant of the family Apocynaceae, they had the same physiological effect (heart arrest), they did not contain nitrogen, both were split by weak acids, they split in the manner of glucosides, and reduced the Fehling liquor. For these reasons he decided to study the former in more detail.[26] First of all, he remarked that the preparation procedure employed by Hardy and Gallois was totally mistaken: they had used alcohol acidified by HCl and, hence, destroyed the largest part of the strophantin present in the raw material. For this reason, he used the following method: The pulverized seeds were extracted with boiling alcohol of 70° in an apparatus provided with reflux. The alcoholic extract was then distilled to eliminate most of the solvent and the concentrated liquid was then further distilled under vacuum. The supernatant oil and resin were separated and the remaining liquid filtrated. The coloration was eliminated with lead sub-acetate and a stream of H_2S. The remaining liquid was evaporated to a syrupy consistency and then left to cool. The precipitated strophantin was purified by successive recrystallization from boiling water. The purified strophantin appeared as white crystalline flakes, hydrated, melting above 100 °C, and having rotatory power α_D = +30° (in concentration 2.3 % in water). It was slightly soluble in cold water, very soluble in alcohol, insoluble in ether, carbon disulfide, and benzene, and precipitated by tannin from its aqueous solutions. Elemental analysis indicated that it contained by weight, 60.46 % carbon, 8.07 % hydrogen, and 31.47 % oxygen, corresponding to the formula $C_{30}H_{48}O_{12}$, thus being the immediate superior homolog of ouabain, $C_{30}H_{46}O_{12}$.[26]

In a following paper Arnaud reported the results of his study of the seeds of Strophantus gratus of Gabon, which were used as arrow poison by the Pahouins in northern Gabon.[27] He extracted the active component using a process similar to the one used with *Strophantus hispidus*. The resulting crystals appeared as very thin lamellae, melting at about 185 °C, highly hydrated (about 17.5 % by weight), water, which they did not lose even in the presence of air dried with sulfuric acid. An aqueous solution of these crystals was levorotatory with α_D = - 33.8° (concentration 6.5 %, 50 °C). In the presence of warm and diluted acids the active substance split into a reducing sugar and an insoluble resin. These properties pointed to a strong similarity between the active substance and ouabain. Elemental analysis of the active component of *Strophantus gratus* indicated that it contained by weight, 58.23 % carbon, 7.71% hydrogen, and 34.06 % oxygen. For this reason, Arnaud repeated the chemical analysis of ouabain. He now reported that the molecular mass of ouabain was 598; at room temperature the substance was present as its heptahydrate, $C_{30}H_{46}O_{12}$, $7H_2O$, and at 100 °C is appeared as the monohydrate $C_{30}H_{46}O_{12} .H_2O$.[27]

In a next paper[28] Arnaud claimed that ouabain and the active principle present in *Strophantus gratus* of Gabon were actually the same substance. He substantiated his claim by reporting that he had found ouabain also in the seeds of *Strophantus gratus* of Gabon and that this ouabain had exactly the same properties and composition as the ouabain present in *Strophantus hispidus.* Hence, the toxicity of *Strophantus gratus* was due to the presence of ouabain.

According to Arnaud, ouabain formed, at least, three hydrates, originating at the temperatures 10° to 20°, 30°, and 60 °C.[29] The first one was a nona-hydrate containing 21.31% of water, assuming that the formula of ouabain was $C_{30}H_{46}O_{12}$. This amount of water decreased to 17.5 % when the hydrate was dried in the presence of sulfuric acid. At the request of Arnaud, the crystallographer Grégoire Wyrouboff (1843-1913) examined the crystalline form of this hydrate and described its characteristics. Arnaud reported that aqueous HCl or sulfuric acid hydrolyzed ouabain into rhamnose and a resin that was certainly a result of the polymerization of the second product of the hydrolysis. Soluble ferments (enzymes), such as emulsin and diastase, had no action upon ouabain. Arnaud also described the action of several reagents upon ouabain, among them, concentrated and diluted nitric acid, bromine, alkalis, acetic anhydride, etc..[29] Further work on the hydrolysis process proved unequivocally that the reducing sugar present in ouabain was rhamnose.[30] The accompanying resin was found to be soluble in hot concentrated alcohol, in methanol, and in ether. Elementary analysis indicated that it contained, by weight, 75.88 % carbon, 7.36 % hydrogen, and 16.76 % oxygen, corresponding to the formula $C_{24}H_{28}O_4$.[30]

Arnaud found that aqueous or alcoholic solutions of the alkalis KOH, NaOH, and $Ba(OH)_2$, even boiling, were unable to hydrolyze ouabain. They actually gave place to a hydrated derivative, without splitting the ouabain. This new substance was found to be a monobasic acid that decomposed the alkaline or terreous carbonates, tinted blue litmus paper, and yielded salts that very soluble in water and non-crystallizable. Arnaud named this new substance *ouabaic acid*. Ouabaic acid was yellow-white amorphous solid, very soluble in water and in alcohol, and insoluble in ether. It melted at about 235 °C with decomposition; it was levorotatory, and decomposed in diluted and boiling acids into rhamnose and a resin very similar to the one originating from the direct hydrolysis of ouabain. Elemental analysis indicated that its composition corresponded to the formula $C_{30}H_{48}O_{13}$. Arnaud reported the preparation and properties of the pertinent salts of sodium, strontium, barium, and lead. [31]

The next paper reported the preparation and properties of a *heptacetyl* derived from the reaction between acetic anhydride and ouabain, in the presence of zinc chloride. [32] This substance was crystalline, insoluble in water, very soluble in acetic acid, dilute or concentrated, very soluble in acetone, soluble in hot alcohol and little in cold alcohol, and insoluble in ether. It melted at about 310 °C with fast decomposition, and was levorotatory. According to Arnaud this acetyl contained still the glucoside function and was a derivative of ouabain and not a product of its splitting. Its elemental analysis corresponded to the formula $C_{30}H_{44}O_{11}$, that is, to the product of dehydrated ouabain with acetic anhydride: $C_{30}H_{37}(C_2H_3O)_7O_{11}$. [32]

Arnaud found that ouabain was completely oxidized by concentrated nitric acid into oxalic acid, with disengagement of CO_2 and formation of a large amount of nitrated amorphous substances insoluble in water. [33] The diluted acid acted in a different manner: no oxalic acid was formed; the oxidation was only partial and accompanied by a hydrolytic process, which generated substances that could be isolated and purified. [33]

Tariric acid

In 1892 Arnaud communicated to the Académie des Sciences the discovery of a new unsaturated fatty acid in the seeds of a plant belonging to the genre *Picramnia Sow*, or *Tariri*, originating from Guatemala. [34] The grains of the plant contained about 67 % of fatty matter easily extractable by means of carbon disulfide. The first extraction produced a slightly yellow fat melting at about 47 °C; the color disappeared upon solution in boiling ether. The fat recrystallized from the extract as beautiful pearly crystals different from all known ordinary fatty material. It was saponified by alkalis, yielding 95 % of a fatty acid (that Arnaud named *tariric acid*) and glycerin in amount corresponding to a triglyceride. The raw acid melted at 49 -50 °C, which after repeated recrystallizations from alcohol of 90o, increased to 50.5 °C. Elemental analysis indicated that it contained, by weight, 76.82 % carbon, 11.68 % hydrogen, and 11.5 % oxygen, corresponding to the formula $C_{18}H_{32}O_2$. Arnaud verified the correctness of the formula by preparing the bromides and the potassium and silver salts. The bromination took place without formation of HBr, indicating the presence of a double or triple bond. The brominated acid,

$C_{18}H_{32}Br_2O_2$, melted at 32 °C and solidified as a crystalline mass, very soluble in chloroform, alcohol, and ether. The tetrabromide was obtained by reacting the acid with the quantity of bromine required by tartaric acid; this was an exothermic reaction accompanied by disengagement of a little HBr from a secondary reaction. Tetrabromotariric acid, $C_{18}H_{32}Br_4O_2$, had a waxy consistency, and melted at 125 °C. Hence the composition of the tariric fat corresponded to the triglyceride $C_2H_3(C_{16}H_{31}O_2)_3$, that is, to the *acetylenic series* $C_nH_{2n-4}O_2$.[34,35]

In a following paper, Arnaud wrote that his new fatty acid was isomeric with the stearoleic acid (9-octadecynoic acid) prepared in 1866 by Otto Overbeck by saponifying monobromoleic acid, $C_{18}H_{32}BrO_2$, or dibromostearic acid, $C_{18}H_{34}Br_2O_2$, with alcoholic KOH.[36,37] At first sight it seemed that these two isomers could be easily hydrogenated, but all the attempts to do so had failed. Arnaud was able to hydrogenate tariric acid by reacting one part of it with ten of HI and a small amount of phosphorus, for six hours at 200 -210 °C in a closed tube. The resulting product was a mixture of fatty acids, melting at 57 °C. After a second recrystallization of the raw mixture from alcohol of 95°, Arnaud obtained 50 g of fatty acids melting at 66 °C. Further numerous recrystallizations from hot alcohol allowed him to obtain an acid melting at 69 °C, the melting point of pure stearic acid. The purity of the acid was determined from the composition of its potassium salt. The same results were obtained when using stearoleic acid.[37]

Tanghinin

Arnaud remarked that much had been written about the biological and physiological aspects of the poison derived from the kernels of the tree Tanghinia venerifera of the Apocynaceae family, growing in Madagascar, but little about its active substance.[38] These kernels contained about 75 % of fatty material that could not be separated by simple pressing. The water contained in the seed formed a hard to break emulsion with this fatty material, which did not allow it to flow easily. Arnaud extracted this material by means of carbon disulfide and treated the solution with boiling alcohol to extract the active substance. Upon evaporation the extract precipitated a crystalline substance, mixed with a number of strongly colored impurities. The latter could be eliminated with common solvents. Arnaud named the active substance tanghinin. Tanghinin was highly toxic, operating as a cardiac poison in a similar manner than ouabain and strophantin, except that it produced a general convulsion state. Pure tanghinin was colorless; its crystals were rhomboid and anhydrous. It melted at about 182 °C and was little soluble in water. After long contact with water it swelled and turned into mucilage. Tanghinin was very soluble in concentrated alcohol and in ether. It was levorotatory with $\alpha_D = -67°$ (20 °C, alcoholic solution); diluted acid turned it into a yellow resinous substance non-crystallizable from alcohol. Elemental analysis indicated that it contained, by weight, 65.79 % carbon, 8.16 % hydrogen, and 26.05 % oxygen. [38]

Digitalin

The strong analogy between tanghinin and digitalin led Arnaud to study the latter in detail. [39] Both substances did not contain nitrogen, where little soluble in water, and very soluble in chloroform and hot alcohol, they were not glucosides because they did not release glucose or other reducing sugar, both resinified under the action of boiling diluted acids, and both were cardiac poisons. Arnaud wrote that pure digitalin appeared as white thin strips, melting at 243 °C, soluble in absolute alcohol and boiling benzene. He submitted this material to a series of successive extractions with alcohol, followed by recrystallization and in every case the melting point of the solid varied between 243 - 245 °C, indicating that digitalin constituted a well defined chemical, and there was no reason to designate the product under the name *digitoxin*, as some chemists had proposed. [39]

In a following publication Arnaud reported that he had determined the composition of digitalin indirectly, from the analysis of its barium salt. He reported that digitalin contained 63.78 % carbon, 8.65 % hydrogen, and 25.57 % oxygen; corresponding to the formula $C_{31}H_{50}O_{10}$ (today we know that the pertinent figures are 60.66 % carbon, 7.92 % hydrogen, 31.42 % oxygen, and $C_{36}H_{56}O_{14}$). Arnaud used the same procedure to determine the composition and formula of tanghinin: $C_{27}H_{40}O_{8}$ (Today we know that the correct composition is 65.06 % carbon, 7.85 % hydrogen, and 27.09 % oxygen, corresponding to the formula $C_{32}H_{46}O_{10}$). [40]

Carotene

Arnaud published as series of papers about the presence of carotene in the leaves of vegetables, its chemical function and formula, dosage, and its probable physiological role. [41,42,43,44,45,46] In his first paper he announced the discovery of carotene in the coloring matter of leaves. [41] He achieved this by separating the coloring matter into its components, by means of successive washes with neutral solvents. Afterwards, he acknowledged that this substance had been mentioned before by Charles Bougarel under the name *erythrophill.* [47] According to Arnaud, the red orange substance appeared as small rhombic crystals, red orange by transparency and blue green by reflection, and melting at 168 °C. It was very soluble in chloroform and carbon disulfide, little soluble in ether, petroleum ether, and alcohol. It was not dissolved and not attacked by water, diluted acids, and alkaline solutions. It dissolved well in concentrated sulfuric acid yielding a violet blue solution. Arnaud proved that this substance was identical with the carotene extracted from carrots, that it was present in the different organs of vegetables, and that it always accompanied chlorophyll. This latter fact seemed to indicated that the two substances where chemically related. [41]

In a following paper Arnaud reported that carotene was also present in many fruits, particularly in tomatoes. [42,43] He now added that carotene oxidized in the presence of air, even at room temperature. This oxidation was very fast with aqueous solutions, particularly at 70 °C. The oxidized product did not have the properties of carotene, thus it was very soluble in cold alcohol, little soluble in carbon disulfide, and melted at 125 °C. Elemental analysis of the oxidized product indicated that it contained, by weight, 70.20 % carbon, 8.60 % hydrogen, and 21.20 % oxygen. Chemical analysis of carotene indicated that it contained, by weight, 88.7 % carbon and 10.6 % hydrogen (for a total of 99.3 %), corresponding to the formula $C_{26}H_{38}$, that is, it was a non-saturated hydrocarbon (today we know that carotene contains 89.49 % carbon and 10.51 % hydrogen, corresponding to the formula $C_{40}H_{56}$). Carotene crystalized as rhombic plates, decomposing above 300 °C under vacuum, and turning into a viscous colorless liquid. Carotene reacted easily with oxygen and the halogens. Arnaud found that 100 g of dry leaves of different plants contained the following amounts in mg, of carotene, using a colorimetric procedure: *Osmunda regalis*, 116.8; *Urtica dioica*, 171.7; *Aesculus hippocastanum*, 118.8; *Tilia sylvestris*, 79.1; *Viola odorata*, 121.0; *Juglans regia*, 118.0; *Ribes rubrum*, 105.5; *Cissus quinquefolia*, 145.1; *Spinaca oleracea*, 160.1; *Vinca major*, 130.0; *Taxus baccata*, 59.5; and *Triticum sativum*, 152.4. [42,43]

In a last paper on the subject Arnaud added another 29 plants to the above list. [44] From these results he observed that the amount of carotene present was higher near or in the flowering period, afterwards it decreased regularly until the fall of the leaves. In addition, the amount of light also influenced the amount of carotene present, the same as with chlorophyll. No carotene was produced in the dark. [44]

Phytocholesterol

It was known that plants contained an immediate principle, isomer of cholesterol, which had the same elemental composition and main properties. Its melting point differed by a few degrees from that of cholesterol and a slightly lower solubility in alcohol. Arnaud wrote that during his work about carotene he had been able to separate the colorless crystalline substance that August Husemann (1833-1877) had separated from carrots and named hydrocarotene. [48] Husemann had assumed that it was a new compound and assigned the wrong composition and formula. Arnaud proved that the product of Husemann was impure and that it still contained carotene. [43] Arnaud submitted his raw material to a long series of purification steps to assure that it did not contain carotene or other impurities. Eventually he obtained a product that melted at 136.5 °C and contained 83.90 % carbon, 12.20 % hydrogen, and 4.31 % oxygen, corresponding to the formula $C_{26}H_{44}O$, exactly that of cholesterol. The same as the latter, this material was insoluble in water, little soluble in cold alcohol and very soluble in boiling alcohol, and also soluble in ether, carbon disulfide, chloroform, petroleum ether, and oils. From alcohol it crystallized as monohydrate, melting at 136.5 °C and losing the crystallization water. From all the non-aqueous solutions it crystallized as anhydrous needles. It was not saponified by alkaline solutions, did not react with dilutes acids, and was levorotatory with α_D = - 35° (chloroform solution). Oswald Hesse (1835-1911) recommended naming it *phytostearin* (today, *phytocholesterol, phytosterol*) to recall its origin and to distinguish it from animal cholesterol. [49]

Natural rubber

Arnaud and Auguste Verneuil (1856-1913) wrote that the rapid growing demand for natural rubber and its derivatives had led to increased efforts to develop the method for extracting the active material from the *Landolphia* vine. Several of the attempts were based in using a chemical extractant, but had failed because they affected the mechanical properties of the rubber. [50] Arnaud and Verneuil developed a mechanical procedure where the barks were first pulverized and then sifted to eliminate most of the fine powder, which was known not to contain rubber. The residue was partially agglomerated by pressure, then soaked in hot water, crushed to transform it into a thick and friable paste, and finally sieved again inside a hot water bath. A second crush of the remaining mass brought to light white vermiform filaments of rubber, which under beating agglomerated into spongy masses containing all the rubber. The product was then throw into water, the rubber floated and the ligneous residue dropped to the bottom. Purification of the rubber was achieved by passing the filaments through laminating cylinders. The overall efficiency for *Landolphia* was very good: 8-9 % for the aerial bark, 14-15 % of the root bark, and 6-8 % for the rest of the vine. [50]

In a following paper Arnaud reported the results of the new extraction method for a variety of different African vines originating from Sudan and Senegal (*Landolphia Heudeloti, Landolphia Senegalensis, and Carpolinus hirsuta*). [51]

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