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Historical Article

The *dextrorotatory sweet asparagine* of Arnaldo Piutti: the original product is conserved in Florence

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Abstract. In 1886, Pasteur presented a note on the work of the Italian chemist Arnaldo Piutti concerning the difference between the two physical isomers (enantiomers) of asparagine. The octahedral crystal of asparagine appeared only as “*levorotatory hemihedralism*” but, in principle, should also exist as a dextrorotatory asparagine with a symmetric crystalline form. In 1886 Arnaldo Piutti isolated the dextrorotatory asparagines while he was working as an assistant of Ugo Schiff in Florence. He obtained also another unexpected information, of which only Pasteur immediately understood the importance: the dextrorotatory aspargine had a sweet taste.
The *dextrorotatory sweet asparagine* of Arnaldo Piutti is conserved in the *Schiff Collection* of the Department of Chemistry “Ugo Schiff” at the University of Florence, and is the first compound where a relationship between the optical *isomerism* of a molecule and a different response of human receptors, in this case the taste, was observed.

Keywords. Asparagine, Chemical Heritage, history of enantioselectivity.

This research stems from a purely museological question. A few years ago, during the reordering of the products synthesized by Ugo Schiff (1834-1915) at that time conserved at the Department of Chemistry “Ugo Schiff” of the University of Florence, and now merged into the Museum of Natural History, a small bottle was found. It carried a “Ugo Schiff” museum label, written by “our” German chemist, referring to: “*Asparagina destrogira dolce*” (*Dextrorotatory sweet asparagine*)[1]. After reading the work by Joseph Gal[2-4] in 2008, Antonio Guarna (the scientific director of the museum project for historical chemical finds) immediately had the intuition that the product could contain the famous dextrorotatory sweet asparagine isolated by Arnaldo Piutti. The historical research that was conducted, within the *Chemical Heritage* project of the University of Florence, proved him right. In this contribution we discuss some insights about the discoverer and how this discovery took place.

"PIUTTI'S ASPARAGINE": WAS IT AN IMPORTANT DISCOVERY?

The work of Gal on the discovery of Piutti reports in the conclusions, the following statements[4]:

> *Piutti was a highly original chemist who carried out notable investigations in a wide variety of research topics. His discovery of a difference in the taste of D- and L-asparagine was a milestone first observation of enantioselectivity at a biological (human) receptor. The discovery was also the first observation of stereoselectivity of any kind in taste; the first finding of biological enantioselectivity in an organism higher than microorganisms; the first example of biological enantioselectivity in an effect other than enzyme action; and one of the two earliest reports of the preparation of a D-amino acid.*

The sample preserved in the *Schiff Collection* of the University of Florence therefore has a considerable historical and scientific relevance. The discovery had a good resonance at the time of Piutti. Louis Pasteur (1822-1895) himself showed a great interest in this research, and in 1886, at the French Academy of Sciences, commented on the work of the Italian chemist with these words[5]:

> *Why this big difference in taste between these two asparagines? One might assume the existence of a very special isomerism, but I think otherwise [...] if two dissymmetrical inverse bodies offer in their interaction with inactive bodies, physical and chemical properties that are very similar and almost identical, these dissymmetrical inverse bodies will give combinations of different absolute properties when they merge with asymmetric and optically active bodies. The active dissymmetrical bodies that will interact with the nervous system, leading to a sweet taste in one case and almost tasteless in another, won't be anything else in my opinion than like the nervous matter itself, dissymmetrical, just like all the basic substances of life: albumin, fibrin, gelatin, etc.*

THE SCIENTIFIC CONTEXT OF ARNALDO PIUTTI'S DISCOVERY

At the end of the Nineteenth century stereochemistry did not exist yet. In 1867 the German chemist Friedrich August Kekulé von Stradonitz (1829-1896) proposed for the first time the tetrahedral structure of carbon. Two years later the Italian chemist and politician Emanuele Paternò (1847-1935) applied this hypothesis to saturated organic compounds[6]. On the relationship between structure and optical properties of a molecule, in 1874, Jacobus Henricus van't Hoff (1852-1911), a Dutch chemist awarded with the Nobel Prize in 1901, proposed that compounds with asymmetric carbon may exist in two different forms corresponding to the two optical antipodes, which could be separated into two optically active compounds. Van't Hoff's theory, considered too audacious and abstract, was almost rejected by the academic world. Only when Hermann Emil Fischer (1852- 1919), a German chemist, almost contemporary of Schiff, and Nobel Prize winner in 1902, in 1894 adduced evidence in favor of this thesis by synthesizing the levorotatory (not natural) antipode of glucose, the propositions of van't Hoff became accepted by the Academics, paving the way to modern stereochemistry[7,8].

ARNALDO PIUTTI BIOGRAPHY

Arnaldo Piutti was born on January 23, 1857 in Udine. He graduated in 1875 at the Technical Institute of Udine, in the Physics-Mathematics section and from there he moved to Turin where he enrolled in the Faculty of Natural Sciences[9]. In 1879 he brilliantly completed his studies under the guidance of Ugo Schiff (1834-1915), a German chemist that served as professor of chemistry in Florence for fifty years and in his Florentine laboratory discovered the *bases* and the *Schiff reaction*[10]. Piutti had the opportunity to meet Schiff just in the two years when the German scientist was lecturing in Turin. In fact, Schiff moved from Florence to Turin in 1877, because of the disagreements he had with the management of the Florentine Royal Institute of Practical and Advanced Studies (*Regio Istituto di Studi Pratici e di Perfezionamento*). He eventually returned to Florence in 1879 after the promise of more funds for his laboratory[11].

After his graduation, Piutti remained in Turin as an assistant to Angelo Mosso and Icilio Guareschi, two important Italian chemists of the time. In 1881 he joined Ugo Schiff in Florence as assistant at the Laboratory of General Chemistry. In Florence, Piutti helped with the course of General Chemistry and later in 1885 also of Organic Chemistry. In 1886 he received a degree in Pharmacy, a professional pratice that was quite usual at that time. In this way he was able to apply for a position in Pharmaceutical Chemistry and in the same year he obtained a professorship at the University of Sassari in Sardinia[9].

In 1888 he moved from Sassari to Naples, where in 1890 he was appointed as full professor, with the maximum score[6] (Figure 1). He also won the position in General Chemistry in Padua, but he preferred to remain in Naples where he could organize the new Institute, following the example of Schiff in the reorganization of the Chemistry Institute Florence[11].

Figure 1. A portrait of Arnaldo Piutti in his laboratory at Naples[9].

Piutti was a member and correspondent of numerous academies and scientific societies, including the prestigious *Accademia dei Lincei*. He held the office of Dean of the Faculty of Science and of the School of Pharmacy in Naples for several years, he became Vice-Rector and represented the Minister of Education at the International Congresses of Applied Chemistry in 1896, 1890, 1900, 1903 and 1910. In Naples he was for a long time appreciated Director of the Institute of Pharmaceutical Chemistry and Toxicology and he personally followed with great commitment the construction of the new headquarters in San Marcellino[9].

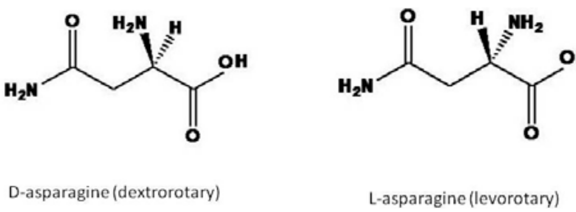
Piutti's scientific contributions are mainly focused on the study of aspartic acid, asparagines and their derivatives and on the optical rotation of organic compounds. His research ranged also in other fields of chemistry. He studied the toxicity of the combustion products of locomotives and he traced the presence of helium in a mineral of the Vesuvian area, demonstrating the diffusion of this gas in solids. He patented *the Pirantina solubile Piutti* (soluble Pirantina Piutti), an antipyretic and analgesic[9].

Despite having personally contributed to the construction of the new laboratories in San Marcellino, he finally abandoned those in the place called "Il Salvatore" only when they were declared unfit. During that period, not being able to carry out experimental work, he devoted himself to theoretical studies on the spatial representation of chemical elements, arranging them in an alternative way to the Mendeleev table[9]. He died in Conegliano, near Treviso, on October 19, 1928.

ISOLATION OF ASPARAGINE

Asparagine is an α-amino acid (Figure 2) that was identified for the first time by the French chemists Louis Nicolas Vauquelin (1763-1829) and Pierre Jean Robiquet (1780-1840) in 1815, in the asparagus sprouts. Shortly afterwards Joseph Bienaimé Caventou (1795-1877), another French chemist who pioneered the research in amino acids and plant alkaloids, and Heinrich Hlasivetz isolated asparagine from *Glycyrrhiza glabra* (liquorice) and *Robinia pseudoacacia*. Later Asparagine was found in many other plant species: in the roots of althea, in potatoes, in hop, in legumes sprouted in darkness and in sweet almonds[12]. To isolate the asparagine molecule, the juice squeezed from the plant was boiled until it formed an abundant coagulation of albumin. At this stage it was filtered, purified and crystallized, obtaining crystals that looked like "sugar candy"[12,13]. The isolated asparagine was an optically active molecule that caused a left-handed rotation of the polarization plane. In 1835 William Hallowes Miller (1801-1880) a British mineralogist and physicist, determined the crystallographic constants and measured its refractive indices.

In 1848 Pasteur identified a relationship between the crystalline form of the asparagine and the rotation of the polarization plane, arguing that in principle, a dextrorotatory asparagine with symmetrical crystalline form should exists, albeit the occurrence of the octahedral crystal of the asparagine only as "*levorotatory hemihedralism*"[14]. Even Karl Friedrich Rammelsburg, (1813-1899), a German mineralogist and chemist, in 1855 advanced the possibility of crystallization in the form of a left or right-handed tetrahedron[15], but the isolation of the dextrorotatory asparagine was carried out only in 1886.

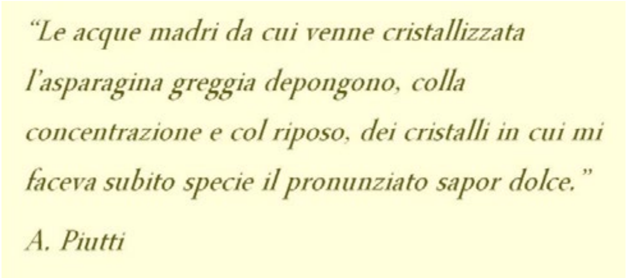


D-asparagine (dextrorotary) L-asparagine (levorotary)

Figure 2. The two forms of asparagine.

THE DISCOVERY OF DEXTROROTATORY “SWEET” ASPARAGINE

Piutti guessed that the failure in identifying the dextrorotatory asparagine was presumably due to its low abundance in Nature. In the factory of Mr. Galgano Parenti, near Siena, Piutti prepared a large quantity of the product from sprouted vetch, a legume[15]. Through fractional crystallization he observed the precipitation of two species of crystals: ordinary levorotatory asparagine, described at that time as “almost tasteless”, and dextrorotatory, which turned out to be “sweet”. Thus the dextrorotatory asparagine could be detected and separated from the levorotatory form on the basis of its taste (Figure 3).



“Le acque madri da cui venne cristallizzata l’asparagina greggia depongono, colla concentrazione e col riposo, dei cristalli in cui mi faceva subito specie il pronunziato sapor dolce.”

A. Piutti

Figure 3. The words of Piutti about the first discover of sweet asparagine: “*From the mother liquor from which the asparagine was crystallized, some crystals formed whose pronounced sweet taste I immediately caught*”[15].

The two types of crystals were analyzed and found to be chemically identical, with the same refractive indices and same plane of optical axes. In the laboratory of Physics headed by Prof. Roiti at the Royal Institute of Practical and Advanced Studies in Florence, Piutti determined the optical rotation of the two species with a Laurent polarimeter, measuring the following values: $[\alpha]_D = -5.43$ for the ordinary or levorotatory asparagine and $[\alpha]_D = +5.41$ for the dextrorotatory sweet asparagine[15] .

The dextrorotatory asparagine, whose existence was theoretically proposed by Pasteur, was finally discovered.

THE SYNTHESIS OF SWEET ASPARAGINE

After separating the dextrorotatory asparagine in 1886, Piutti succeeded in setting up the synthesis in hislaboratory in 1887[16]. The synthesis was carried out through the reduction of the oxime of oxaloacetate ether. Piutti separated two monoethyl esters, written with the formulae: $COOC^2H^5$-CH-$CHNH^2$-COOH and $COOC^2H^5$-$CHNH^2$-CH^2-COOH (according to the original notation). From the former derivative he obtained the amidate, and finally the two rotatory asparagines, identical to the natural products and called by Piutti as a whole “β-asparagine”, with the formula: $CONH^2$-CH^2-$CHNH^2$-COOH (Figure 4). This is of course a historical nomenclature, which does not comply with the common rules currently in use. From the second amidate he obtained the inactive “α-asparagine”, an isomeric form of the β-asparagines. Thus, he came to the conclusion that he had obtained *three asparagines*. He did not know whether the inactive form, called “α-asparagine”, could be separated into two optically active asparagines.

He successfully separated the β-asparagines and found that the compounds, like the natural products, differed in the rotation of the plane of polarization as well as in taste, in solubility and in density. Piutti wrote:

> *The synthetic asparagines, thus obtained, differ from each other, just like the natural ones. Besides the different hemihedralism and the opposite rotatory power, even the taste, which in the levorotatory is insipid, while the dextrorotatory is “sweet”. Furthermore “…as the rotatory asparagines have the same chemical composition, they are to be considered as physical isomers.*

Piutti continued:

> *This result […] also shows how the dextrorotatory asparagine, discovered by me in the vetch and now obtained by synthesis, is the physical isomer of the ordinary asparagine*[15].

According to Piutti “*the second* [amidate] *supplied the inactive species, chemically isomeric, and until today unknown*” (inactive α-asparagine) that he specified with

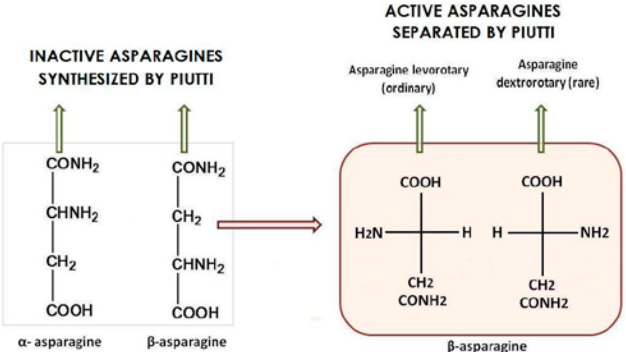


Figure 4. Nomenclature given by Piutti to the “asparagines” he found: on the left the inactive asparagines that he called α-asparagine and the “mixture” of the two optically active asparagines, that he called β-asparagine (α-asparagine and β-asparagine are two racemic mixtures; Piutti reported this observation, but was not able to confirm this assumption). On the right the products obtained by separation of the mixture of β-asparagine: the asparagine levorotatory, tasteless, and the dextrorotatory, with a sweet taste.

the formula: CONH^2-CHNH^2-CH^2-COOH. He concluded by saying:

> *I intend to complete the study of this asparagine* [inactive α-asparagine] *and to determine whether or not it is separable from the two rotatory asparagines of the same composition (such as the presence of an asymmetric C would suggest) when I will be able to prepare it in greater quantities.*

In 1890 Piutti modified the preparation method [17]. This time the asparagines were obtained from the silver salt of "*acido γ-ossimmidosuccinico*" in ether via solvent evaporation and filtration of the iodide excess. The result is an oil: "*nitrilosuccinato dietilico*", a nitrogen derivative of diethyl succinate. The oil was treated with bromine in acetic acid solution yielding the compound $C_6H_7N_2O_3Br$ which is optically inactive. This compound was reduced in acetic acid solution with sodium amalgam. The mother liquor was left resting for a long time together with copper acetate; finally:

> *with a fine sieve the* [inactive] *α-asparagine, reduced to powder by the loss of its crystallization water, is separated from the crystals of the rotatory β-asparagines, in their turn recognizable and separable by their taste or by their different hemihedralism*[17].

In this way Piutti understood that the formation of the rotatory asparagines is independent on the synthesis procedure. This observation also emphasizes the instability of the inactive asparagine for what he refereed to as "reasons of physical order". However, his goal changed: he was prompted to isolate the inactive α-asparagine, and to separate it into the two rotatory forms. He wrote:

> *The resolution of the inactive α-asparagine into two corresponding rotatory asparagines acquires therefore more interest now and I am confident of having the means to experiment later*[17].

PRESENCE IN NATURE OF THE SWEET FORM

In 1915 Piutti made another important discovery: the two rotatory β-asparagines "*coexist in the products of germinated lupins*" [18]. According to Piutti, as the dextrorotatory sweet asparagine is mainly used by the plant itself, much more than its optical antipode, only a small quantity can be isolated. Finally the dextrorotatory asparagine disappears with the process of germination[18].

The presence of dextrorotatory sweet asparagine was not due to racemization during the extraction from the plant, but according to Piutti's hypothesis, it was already present in the plant itself. The dextrorotatory sweet asparagine is the D-aminoacid: Piutti demonstrated its presence in nature.

During the years in which the theories of van 't Hoff on optically active compounds were not yet easily accepted, Piutti successfully identified the two enantiomers of asparagines. He demonstrated the correspondence between the crystallographic form and the optical properties of a chiral molecule and discovered a correlation between the different optical rotation of the molecule and the different response of the human receptors[17.] Finally, a few years later, he observed the enantio-specific use of amino acids by plants [18].

The existence of the two rotatory asparagines in lupins and their selective use in the plant and the observation that only one of the two enantiomers interacts with our receptors, giving the sweet taste, represented two major discoveries in biological chemistry. These results were of fundamental importance that, perhaps Piutti (unlike Pasteur) did not fully understand.

THE ASPARAGINE OF PIUTTI IS CONSERVED IN THE MUSEUM OF NATURAL HISTORY IN FLORENCE

The original sample of *dextorotary sweet asparagine* obtained by Piutti in 1886 is conserved in the *Schiff Collection* of the University of Florence.

Recently, the sample of *dextrorotatory sweet asparagine* has been included as a cultural asset to the scientific-technological heritage ("PST", *Patrimonio Scientifico e Tecnologico*), according to the Italian Ministry for Cultural Heritage and Activities (*Ministero per i Beni e le Attività Culturali*). The information concerning this sample are available online through the SigecWeb system of the Central Cataloging and Documentation Institute (ICCD)[19]. The scientific and private correspondence of Ugo Schiff is also conserved at the "Ugo Schiff" Department of the University of Florence, including the correspondence between Schiff and Piutti of those years. A study of these letters will elucidate how and why the sample of the asparagine of Piutti remained in Florence in his master's laboratory. Furthermore, it can shed light on Piutti's arguments related to his experimental work and whether he really understood or not the importance of those discoveries.

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Figure 5. The *dextrorotatory sweet asparagine* of Piutti: on the left the compound in the original bottle with the original label handwritten by Ugo Schiff; on the right, a particular of the crystals of original asparagines contained in the bottle. This item is conserved in the *Schiff Collection* of "Ugo Schiff" Chemistry Department at University of Florence (class: *Collezione Schiff*, DCO20). Courtesy of the Museum of Natural History of the University of Florence.