Scientific Research Publishing

American Journal of Analytical Chemistry, 2023, 14, 229-237
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Determination of Atomic Fractions of Isotopes Carbon-13 and Nitrogen-15 Directly in Glicine, L-Leucine, Isoleucine and Alanine

Levani Eliashvili*, Lamzira Parulava, Vakhtang Betlemidze

National High Technology Center of Georgia, Tbilisi, Georgia
Email: *levani_eliashvili@gtu.ge

How to cite this paper: Eliashvili, L., Parulava, L. and Betlemidze, V. (2023) Determination of Atomic Fractions of Isotopes Carbon-13 and Nitrogen-15 Directly in Glicine, L-Leucine, Isoleucine and Alanine. *American Journal of Analytical Chemistry*, **14**, 229-237.
https://doi.org/10.4236/ajac.2023.145013

Received: November 8, 2022
Accepted: May 28, 2023
Published: May 31, 2023

Abstract

Using compounds modified by the isotopes carbon-13 and nitrogen-15 helps research in various fields of science, such as medicine, pharmacology, pharmacokinetics, metabolism, agriculture, and others. In case of the availability of reliable, express, and cheap methods, the area of their use will gradually expand. A determination of the atomic fraction of the isotopes carbon-13 and nitrogen-15 directly in glycine, leucine, isoleucine, and alanine is proposed; the modification concerns all centers or one or more identical carbon and nitrogen centers separately, as well as both isotopes at the same time. There are defined mass lines of the mass spectrum of each amino acid, through which the isotopic content of carbon and nitrogen is calculated. The processes that must be taken into account for the determination of the isotopic content are also established. Isotopic analysis of these compounds until now was carried out by transforming them into carbon oxide, dioxide, and molecular nitrogen, and determination of their content in individual centers was impossible.

Keywords

Mass Spectrometer, Mass Spectrum, Fragmentary Ion, Ionization, Mass-to-Charge Ratio, Intensity of Ionic Current, Carbon-13, Nitrogen-15, Atomic Fraction, Isotope-Modified

1. Introduction

The nomenclature of compounds modified by the isotopes carbon-13 and nitrogen-15 is gradually expanding and the area of their use is gradually expanding. Amino acids are the most important compounds for life and so much attention is paid to their metabolism in the human body. Several studies have been conducted

and their metabolic routes have been proposed. The use of isotope-modified amino acids will help determine their metabolism in the human body accurately and definitively, and at the same time control the processes of their synthesis [1]. Variations of these elements are expected to occur during biosynthesis; determination of these variations, in turn, is also very important.

Mass spectra of these substances have been studied, but some secondary processes, which must be taken into account for mass spectrometric analysis directly in these compounds, have not been recorded [2]-[7]. It is impossible to carry out isotopic analysis without taking them into account. The availability of simple, express, and reliable methods will make it possible to use these compounds in the future in various scientific studies, the solution of which was impossible without the use of isotope-modified amino acids.

As is known, the compounds modified by the isotopes carbon-13 and nitrogen-15 for conducting the mass-spectrometric analysis are converted into compounds "convenient" for mass-spectrometric analysis: carbon oxide or carbon dioxide and molecular nitrogen. Amino acids can be isotope-modified by one or more carbon or nitrogen centers. The isotopes of carbon-13 and nitrogen-15 may have different atomic fractions in different isotopic centers. They cannot be determined by the conversion method, information about the isotopic content in separate centers is lost, and only the average isotopic content of carbon and nitrogen in the analyzed compound is determined. The authors of the papers [2]-[7] have proposed a mathematical model of the possibility of direct (immediate) mass spectrometric analysis of researched organic compounds. In the work [2], as well as in the works [2]-[7], a mathematical model for determining the isotope nitrogen-15 was proposed. Specific cases of their use in some compounds are given. The mass spectra have been studied by the proposed approach in glycine, L-serine, threonine, methionine, leucine, isoleucine, glutamic acid, valine, proline, alanine, asparagine, aspartic acid, and glutamine. The processes that must be taken into account for the determination of the isotopic content in them have been determined and the formulas are given.

2. Experimental

➢ The mass spectrum of glycine is given in **Figure 1**

Even though the molecule has two highly polar groups and has a short chain, the molecular peak in the spectrum is intense compared to other amino acids. The NH_2 group is the most electronegative in the molecule, so the charge is mainly localized on the nitrogen atom, and therefore the amine type fragmentation occurs here, and it has the most intensive peak m/z = 30 (CH_2-NH_2+) in the spectrum, and therefore the peak of ions with m/z = 45 is less intensive. During fragmentation, water is also eliminated by the migration of hydrogen from the amine group (low-intensity ions m/z = 57, 58), the spectrum also shows ions m/z = 60, 61, which are formed by the migration of hydrogen to the carbon atom and subsequent fragmentation from the amine group. The migration of hydrogen

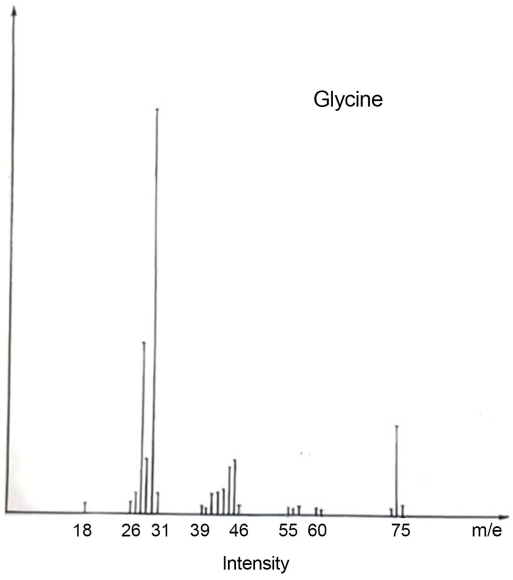

Figure 1. The mass spectrum of glycine.

from the amine group to the carboxyl group and cleavage of CO_2 also occur and ions m/z = 31 are formed.

The isotopic content of carbon in glycine isotopically modified by both centers is determined by molecular peaks. The conducted research has shown that in this case, we should take into account both the formation of molecular peaks and fragmentation by breaking off the hydrogen atom. The formula that can be used to calculate the ratio $Y = \frac{X_{^{13}\mathrm{C}}}{X_{^{12}\mathrm{C}}}$ is as follows:

$$3I_{74}Y^2 - \left(2I_{75} - 0.01030I_{74}\right)Y + \left(I_{76} - 0.00515I_{75} - 0.00405I_{74}\right) = 0 \qquad (1)$$

where I_{74}, I_{75}, I_{76} are respectively the intensities of the peaks with m/z = 74, 75, 76, and $X_{^{13}\mathrm{C}}$ and $X_{^{12}\mathrm{C}}$ are the atomic fractions of carbon-13 and carbon-12, respectively. The numerical coefficients (in all equations and formulas used in the article) take into account the natural distribution of heavy isotopes of other elements entering the molecule. The atomic fraction of the carbon-13 isotope in percent is calculated using the formula:

$$X_{^{13}\mathrm{C}}\% = \frac{Y}{Y+1} \times 100\% \qquad (2)$$

It is also possible to calculate the ratio of isotopic content of carbon by ion peaks with m/z = 29, 30, 31. The equation that determines the Y ratio of carbon isotopes has the following form:

$$I_{29}Y^2 - \left(I_{30} - 0.00837I_{29}\right)Y + \left(I_{31} - 0.00426I_{30} - 0.01141I_{29}\right) = 0 \qquad (3)$$

The same equation can be used to calculate Y when glycine is isotope-modified by an α carbon center. If glycine is isotope-modified by the carbon of the carboxyl group and the α carbon center is of natural distribution, then Y is determined by the following equation:

$$I_{74}Y^2-\left(I_{75}-0.01635I_{74}\right)Y+\left(I_{76}-0.01635I_{75}-0.00385I_{74}\right)=0 \quad (4)$$

When the content of carbon isotopes in the carbon centers of glycine is not the same, we have in the equation two unknowns Y' and Y'', and the equation is reduced to the following form:

$$\begin{cases}\left(Y'+Y''\right)^2 I_{74}-\left(I_{75}-0.00516I_{74}\right)\left(Y'+Y''\right)-I_{74}Y'Y''+\left(I_{76}-0.00516I_{75}-0.00405I_{74}\right)=0\\ 0.00516\left(Y'+Y''\right)^2 I_{74}-\left(0.00516I_{75}-0.00003I_{74}\right)\left(Y'+Y''\right)-0.0050I_{74}Y'Y''+\left(I_{76}-0.00408I_{75}\right)=0\end{cases} \quad (5)$$

where $Y'=\frac{X_{^{13}\mathrm{C}}}{X_{^{12}\mathrm{C}}}$ in the α carbon center, and $Y''=\frac{X_{^{13}\mathrm{C}}}{X_{^{12}\mathrm{C}}}$ in the carbon center of the carboxyl group. It is possible to determine the isotopic content of carbon in both carbon centers simultaneously in a simpler way.

Y in the α-carbon center should be determined by the formula (3) and then by formula (4), where the isotopic content of the α-carbon center will be taken into account by numerical coefficients. The content of the isotope carbon-13 in natural glycine determined by these methods is given in Table 1.

For determination of atomic fractions of the isotope of nitrogen intensities of the mass lines with m/z = 74, 75, 76 also are used. $Y=\frac{X_{^{15}\mathrm{N}}}{X_{^{14}\mathrm{N}}}$ is calculated by the equation

$$I_{74}Y^2-\left(I_{75}-0.02374I_{74}\right)Y+\left(I_{76}-0.02389I_{75}-0.00494I_{74}\right)=0 \quad (6)$$

The atomic fraction of the isotope nitrogen-15 in percent is calculated by the formula:

$$X_{^{15}\mathrm{N}}\%=\frac{Y}{Y+1}\times 100\% \quad (7)$$

Table 1. The content of the isotope carbon-13 in natural glycine.

No.	The atomic fraction of the isotope carbon-13, calculated by the formula		
	No. 1	No. 3	No. 4
1	1.15	1.14	1.15
2	1.15	1.11	1.13
3	1.16	1.15	1.11
4	1.09	1.13	1.09
5	1.10	1.12	1.10
6	1.10	1.12	1.09
7	1.12	1.12	1.10
8	1.14	1.14	1.14
9	1.10	1.14	1.12
10	1.09	1.11	1.16
	1.11 ± 0.02	1.13 ± 0.02	1.12 ± 0.02

The atomic fraction of the natural content of nitrogen-15 by the given method has been measured—0.37%.

➢ The mass spectrum of leucine is given in **Figure 2**

In the mass spectrum of leucine, the molecular peak is not observed because leucine has a branched structure and its fragmentation occurs during an electron bombardment, an intensive peak with m/z = 86 is in the mass spectrum, it is formed as a result of the cleavage of the carboxyl group and contains both carbons of the main chain and nitrogen, but does not contain carbon of the carboxyl group. Further dissociation occurs with a four-step migration of the hydrogen atom and ions with m/z = 30 ($H_2C = NH_2$) and ions with m/z = 44 ($H_2C = CH\text{-}NH_3$) are formed, and a six-step rearrangement also occurs (the so-called McLafferty rearrangement); ions with m/z = 75 ($NH_2\text{-}CH\text{-}C(OH)_2$) and also m/z = 57 ($NH_2\text{-}CH\text{-}C\text{-}O$) are formed.

Fragmentation with the breaking of the α bond is a less probable process, therefore the ions m/z = 74 are of low intensity ($NH_2\text{-}CH\text{-}COOH$); the breaking of the β bond is also less probable (ions m/z = 43). The isotopic analysis of carbon, when leucine is modified by carbons of carbon chains or all centers are equally modified, can be determined by the formula:

$$X_{13_C}\% = \left(\left(I_{87} - 0.00544 I_{86}\right) / \left(I_{87} + 4.99456 I_{86}\right)\right) \times 100\% \quad (8)$$

It is possible to determine the atomic fraction of the isotope carbon-13 by the ions whose m/z = 74, 75, 76. In this case, it is enough to take into account two processes, the separation of the fragment $(CH_3)_2CH\text{-}CH_2$ from the molecular ion and the so-called McLafferty rearrangement, and is determined by Equation (1). If leucine is modified by carbon centers other than the carboxyl group, then the isotopic content of carbon is determined by the same equation. And the isotope content of carbon in the carboxyl group can be determined by Equation (4). When leucine is isotope-modified non-uniformly by two carbon centers, then the system of equations will be reduced to the system (5).

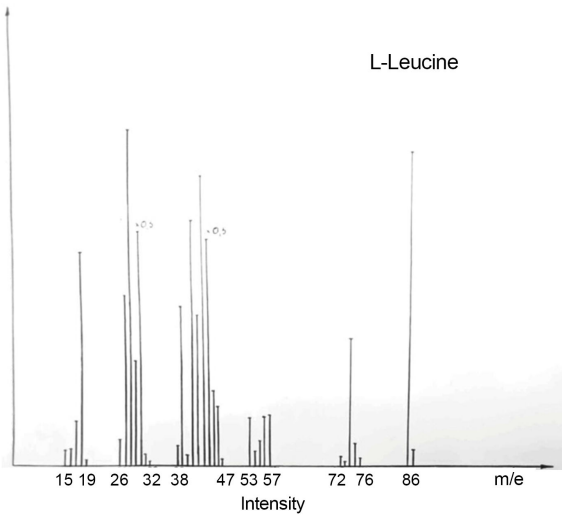

Figure 2. The mass spectrum of L-leucine.

Results of measurement of the natural distribution of the isotope carbon-13 in leucine are given in Table 2.

In leucine, isotope-modified by the isotope of nitrogen, the atomic fraction of the isotope nitrogen-15 is determined by the mass lines m/z = 86, 87

$$N^{15} = \left(I_{87} - 0.05780 I_{86} / 0.94220 I_{86} + I_{87}\right) \times 100\% \quad (9)$$

For the determination of atomic fractions of the isotope of nitrogen the intensities of the mass lines with m/z = 74, 75, 76 also are used through the formula (6).

➢ The mass spectrum of isoleucine is given in Figure 3

Table 2. Results of measurement of the natural distribution of the isotope carbon-13 in leucine.

No.	The atomic fraction of the isotope carbon-13, calculated by the formula		
	No. 1	No. 3	No. 4
1	1.11	1.14	1.12
2	1.12	1.13	1.13
3	1.10	1.12	1.11
4	1.10	1.10	1.13
5	1.11	1.14	1.12
6	1.15	1.13	1.13
7	1.12	1.09	1.10
8	1.08	1.14	1.14
9	1.12	1.13	1.12
10	1.13	1.15	1.16
	1.11 ± 0.02	1.13 ± 0.02	1.13 ± 0.02

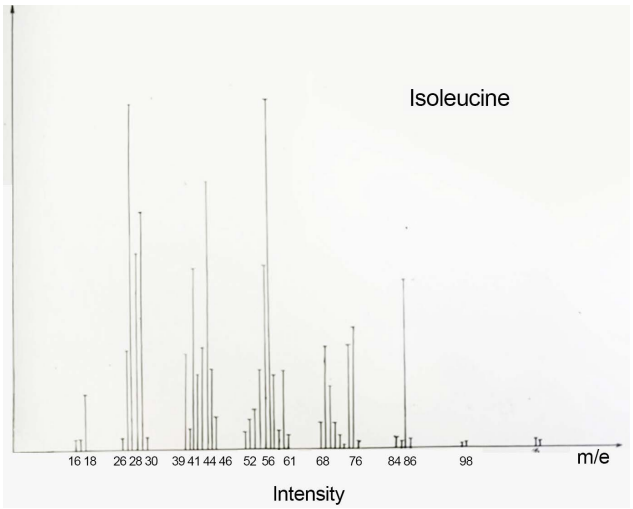

Figure 3. The mass spectrum of isoleucine.

Molecular lines are not recorded in the spectrum. The charge is mainly localized on the amine group and therefore the fragments containing amine groups are intense. During fragmentation, intensive rearrangement takes place, this is confirmed by the peaks with m/z = 30, 44, 57, 75. The ions with m/z = 75 $(NH_2–CH–C–(OH)_2)+$ are formed by rearrangement. These ions are more intensive than the ions with m/z = 74 $(NH_2–CH–C–OOH)+$ obtained by alpha cleavage.

The peak with m/z = 57 $(NH_2–CH–C=O)+$ is also intensive, but there is partial superposition with the ions—$(NH_2–CH–CH–CH_3)+$. In isoleucine, the four-step rearrangement is less probable than in leucine, so the peaks of ions with m/z = 30, 44 are more intensive. The decarboxylation process is energetically more favorable for the molecule of isoleucine, and ions with m/z = 86 are formed; at the same time, the less probable decarboxylation process is observed. There are fewer probable fragments in the spectrum with m/z = 114, 99.

A study of direct isotope analysis in isoleucine shows that the atomic fraction of carbon in isoleucine, modified by all carbon centers, is carried out through masses of m/z = 86, 87; when the atomic fraction of carbon-13 isotope is more than 1% it is calculated by the formula (8). And when the atomic fraction is less than 1%, it is necessary to take into account the hydrogen abstraction, then the atomic fraction of the isotope carbon-13 is determined by the intensities of the mass lines with m/z = 85, 86, 87 and the ratio of isotopes is calculated by the following equation:

$$15I_{85}Y^2 - \left(5I_{86} - 0.02720I_{85}\right)Y + \left(I_{87} - 0.005440I_{86} + 0.00003I_{85}\right) = 0 \qquad (10)$$

The isotopic fraction of carbon-13 can be calculated using formula (4), but in the samples with natural distribution, it gives us a higher result than the real one, so the process of hydrogen abstraction from this fragment should be taken into account, in this case, the system of equations will be reduced to a cubic equation, which complicates the calculations. The calculation results are given in **Table 3**.

Table 3. The calculation results of The isotopic fraction of carbon-13.

No.	The atomic fraction of the isotope carbon-13, calculated by the formula	
	No. 8	No. 4
1	1.13	1.75
2	1.12	1.81
3	1.10	1.90
4	1.12	1.82
5	1.10	1.81
6	1.13	1.75
7	1.14	1.78
8	1.10	1.83
9	1.12	1.84
10	1.13	1.76
	1.12 ± 0.02	1.81 ± 0.04

The atomic fraction of the isotope nitrogen-15 is calculated by the formula (9) when the content of the isotope nitrogen-15 is greater than 2%; and when it is less than 2%, it is calculated by the formula (11).

$$I_{85}Y^2-\left(I_{86}-0.05780I_{85}\right)Y+\left(I_{87}-0.05780I_{86}+0.00198I_{85}\right)=0 \qquad (11)$$

The natural content of the isotopes of nitrogen in the samples of isoleucine is obtained at 0.38%.

➢ Mass spectrum of alanine, Figure 4

In the spectrum, the peak of ions with m/z = 44 is the maximum. This fragmentary ion is formed by alpha cleavage and abstraction of the carboxyl group; as in the rest of the amino acid, the charge is mainly localized on the NH_2 group. The alpha cleavage from the other side is relatively less intensive and ions with m/z = 73, 74, 75 are formed. As for the molecular ion, it is very unstable and not recorded in the spectrum even at a trace level, the conducted experiments showed us that direct mass spectrometric analysis is preferable to be performed according to the intensity of the peaks with m/z = 73, 74, 75; in this case, we consider only the loss of the methyl group and hydrogen migration to the amine group with the further abstraction of CH_2 and H. The equation that can be used to determine the atomic fraction of the isotope carbon-13 in fully modified alanine is as follows:

$$3I_{73}Y^2-\left(2I_{74}-0.01002I_{73}\right)Y+\left(I_{75}-0.00501I_{74}-0.00406I_{73}\right)=0 \qquad (12)$$

whereas isotopic concentration is calculated by the formula (2).

The content of the isotope of carbon in alanine can also be calculated according to the isotope-modified centers. If it is isotope-modified only by the carbon of the carboxyl group, the ratio of carbon isotopes is calculated by the equation:

$$I_{73}Y^2-\left(I_{74}-0.01621I_{73}\right)Y+\left(I_{75}-0.01621I_{74}-0.00385I_{73}\right)=0 \qquad (13)$$

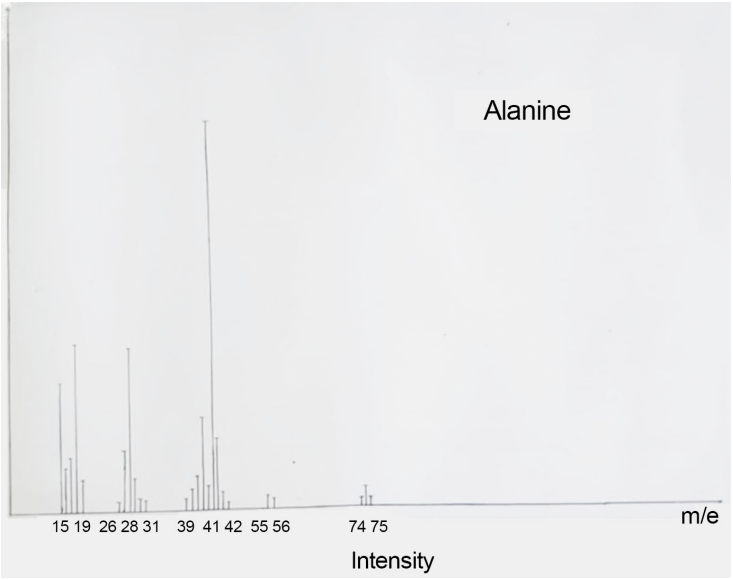

Figure 4. Mass spectrum of alanine.

In the case when alanine is modified not only by the carboxyl carbon but also by the rest of the carbon atoms, both calculations are relatively difficult and it is necessary to solve the system of equations:

$$\begin{aligned}&\left(Y'+Y''\right)^2 I_{73}-\left(I_{74}-0.00501I_{73}\right)\left(Y'+Y''\right)-I_{73}Y'Y''+\left(I_{75}-0.00501I_{74}-0.00406I_{73}\right)=0\\&0.00501\left(Y'+Y''\right)^2 I_{73}-\left(0.00501I_{74}-0.00002I_{73}\right)\left(Y'+Y''\right)-0.00486I_{73}Y'Y''+\left(I_{76}-0.00408I_{75}\right)=0\end{aligned}\quad(14)$$

The atomic fraction of the isotope carbon-13 is calculated by the formula (12) and is equal to 1.21, with an accuracy of ±0.02.

The atomic fraction of the isotope nitrogen-15 in alanine can be determined similarly by m/z = 73, 74, 75; at this time, we take into account the following processes: the formation of fragmentary ion and abstraction of hydrogen from the fragmentary ion. The equation that determines the isotope ratio of nitrogen has the following form:

$$I_{73}Y^2-\left(I_{74}-0.057797I_{73}\right)Y+\left(I_{75}-0,02374I_{74}-0.00368I_{73}\right)=0\quad(15)$$

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Koga, T. and Naraoka, H. (2022) Synthesis of Amino Acids from Aldehydes and Ammonia: Implications for Organic Reactions in Carbonaceous Chondrite Parent Bodies. *ACS Earth and Space Chemistry*, **6**, 1311-1320. https://doi.org/10.1021/acsearthspacechem.2c00008

[2] Parulava, L., Eliashvili, L., Betlemidze, V. and Mzareulishvili, N. (2022) Determination of Atomic Fraction of Isotope Carbon-13 Directly in Urea, Benzophenone, Nitrobenzene, Benzoic Acid and 2-Hydroxybenzoic Acid. *American Journal of Analytical Chemistry*, **13**, 195-205. https://doi.org/10.4236/ajac.2022.136014

[3] Parulava, L., Eliashvili, L., Betlemidze, V. and Mzareulishvili, N. (2022) Mass-Spectrometric Method of Measurement of Isotopic Content of Nitrogen in Organic Compounds. *American Journal of Analytical Chemistry*, **13**, 186-194. https://doi.org/10.4236/ajac.2022.135013

[4] Orjonikidze, K.G., Kerner, M.N., Parulava, L.P. and Osier, Yu.P. (1986) Isotopic Analysis of Carbon in Organic Compounds on the II-III Class Mass-Spectrometers.

[5] Orjonikidze, K.G., Kerner, M.N. and Parulava, L.P. (2001) The Method of Mass-Spectrometric Determination of Isotopic Concentration of Carbon in Methanol. Inventor's Application, No. 3673578. (In Russian)

[6] Jaliashvili, V.L., Vakhania, G.V., Melitauri, N.K., Orjonikidze, K.G. and Parulava, L.P. (1989) The System of Direct Insertion of Solid Samples into Mass Spectrometer МИ-1201. *PTE*, **3**, 182-183. (In Russian)

[7] Momigny, I. (1956) Sur la structure de la pyrite. *Bulletin de la Société Royale des Sciences de Liège*, **25**, 93.