The Synthesis and Physico-chemical Characterization of Anthocyan-magnesium Compound

CERASELA ELENA GÎRD*, LIGIA ELENA DUŢU, TEODORA DALILA BALACI, VERONICA NACEA
"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy 6, Traian Vuia Str., 020956, Bucharest, Romania

This paper presents the results of the studies regarding the obtaining and the physico- chemical characterization of the semi-synthesis compound called anthocyan-magnesium. The coupling of the anthocyans extracted from Ribes nigrum L. (black currant) with magnesium had the purpose to sum the therapeutic properties of the two components. The anthocyanic extract contains up to 22.4 – 24.0% anthocyans (expressed in cyanidin chloride). The anthocyan-magnesium compound contains 13.37 – 15.25% anthocyans, 11.23 – 11.29% magnesium ions (14 mEg) and 1.56 – 1.96% potassium ions. Spectral methods (IR and UV-VIS spectroscopy) and magnetic determinations confirm the structure of the semisynthetic compound. The coupling of the anthocyans extracted from Ribes nigrum L. (black currant) with magnesium had the purpose to sum the therapeutic properties of the two components. The anthocyanic extract contains up to 22.4 – 24.0% anthocyans (expressed in cyanidin chloride). The anthocyan-magnesium compound contains 13.37 – 15.25% anthocyans, 11.23 – 11.29% magnesium ions (14 mEg) and 1.56 – 1.96% potassium ions. Spectral methods (IR and UV-VIS spectroscopy) and magnetic determinations confirm the structure of the semisynthetic compound.

Keywords: anthocyans, anthocyan-magnesium

The option for antocyanosides (derivatives of 2-fenyl-bensopyrilium) is supported by their interesting pharmacological properties, such as: protecting the capillaries, kelatation of the super-oxide free radicals released in various pathological states, decreasing the permeability of the haemato-encephalic barrier for toxic substances, increasing visual aquity, inhibiting the lymphopenisant action of cyto-static drugs [1,2].

The association with magnesium took into account the frequent use of this ion (as salts) as an anti-spastic, as well as its involvement at the level of the different fosphatase enzymes [2, 3].

The combination of antocyanosides with the active biological ion had the purpose to "ennoble" it by cumulating the specific actions of anthocyanosides with that of the cation. Thus, the resulting compound widens the range of uses of anthocyanosides to include the field of oligo -therapy.

Experimental part

The vegetable raw materials are the fresh fruits of black currant (Ribes nigrum L.).

The research followed several steps, as follows:

- obtaining the „antochyanosidic total extract" by hydro-alcoholic fermentation of the freshly crushed fruits, in the presence of Saccharomices cerevisiae;
- standardizing the extract obtained, by identifying and assaying the active principles responsible for the therapeutic effects [4];
- synthetizing the compound by directly coupling the magnesium oxide (decarbonated and activated through repeated calcinations) with the extracting solution standardized in anthocyanidosides, in an acid environment with a ratio of 2:1 [5];
- bringing the compound in a solid state by concentrating the solution at low pressure;
- stabilizing the structure of the resulting compound through spectroscopy (IR, UV-VIS) and measuring the magnetic susceptibility.

The quantitative determination of active principles

The anthocyanosides were assayed through a spectrophotometric method, based on the measurement of the absorbance of the anthocyanosides in a slightly acid medium (*p*H=5,0), at 520 nm, using a 20 g/L solution of hydrochloric acid as the compensation liquid [2,4]. The measurements were performed on a /VIS–V 530 spectrophotometer.

The magnesium in the semi-synthesis compound was assayed through a complexonometric method - direct titration with complexone III, using black eriocrom T as indicator [6]. Because the anthocyanosides can interfere with the turning of the indicator, the organic matter was removed through calcination, and the resulting magnesium oxide was made soluble in hydrochloric acid.

Potassium was assayed through a gravimetric method, based on Na ($B(C_6H_5)_4$) precipitation [6]. In order to remove the organic matter, the sample was first disintegrated with concentrated sulphuric acid and hydrogen peroxyde and the residue was solubilized in water.

Spectral description of the anthocyan-magnesium

The IR absorption spectres were recorded on a SPECORD IR 71 C ZEISS Jena apparatus, on the domain 4600-6550 cm^{-1}, using the method with potassium bromide. This method is based on the elasticity of the chemical substance not on very high pressures as well as on the fact that it does not have 400-5000 nm absorption bands [7]. The cyanidol chloride was used as standard substance, with a concentration of $2.19 \cdot 10^{-5}$ M, in carbon tetrachloride.

The electronic spectres were recorded on a SPECORD M400 Carl Zeiss Jena spectrophotometre in the 50,000-11,000 cm^{-1}, using a type 45/0 device for diffuse reflection, for powders (as dillution matrix MgO of spectral purity was used).

In order to determine the magnetic susceptibility, the Faraday scales were used. Work environment: 17 ^{0}C, scales calibration with $Hg\,Co\,(SCN)_4$ [2,8].

* *email: cerasela.gird@ yahoo.com; tel:(+40) 021 3180746*

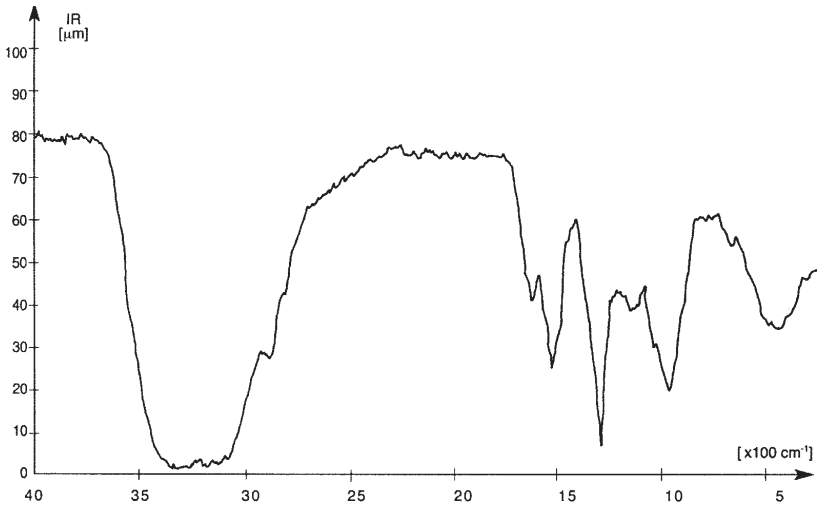

Fig. 1. IR spectre of the anthocyanosidic extract

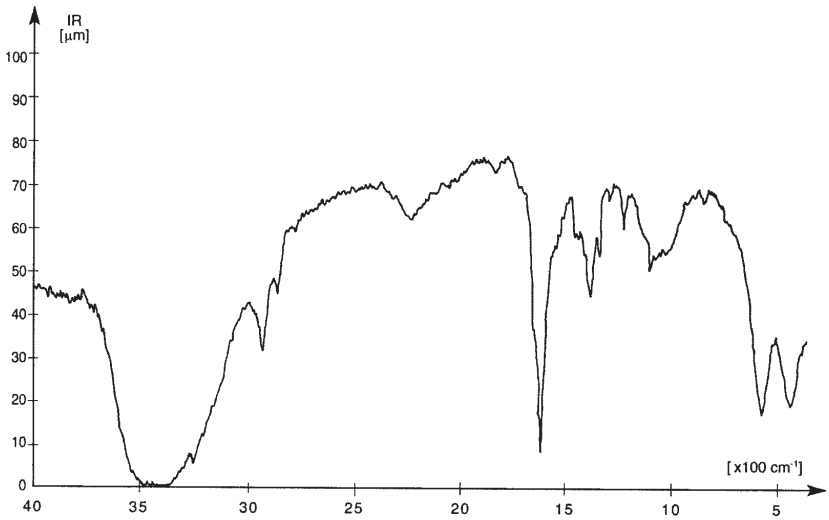

Fig. 2. IR spectre of the cyanidin chloride

Results and discussions

The extract obtained following the hydro-alcoholic fermentation is clear, with a red-brown color, a particular smell, astringent sweet-bitter taste and contains proantocyanidines (3.4-dihidroxy-flavan derivates), flavones (2-fenyl-γ–benzopyrone derivates), catechic tannins (3-hidroxy-flavan derivates) and 22.0-24.0 g% total anthocyanosides (expressed in cyanidin chloride).

The coupling-compound (in a solid state) is a hygroscopic crystalline compound, red-brown, soluble in water, 20 g/L hydrochloric acid and alkaline solutions. It contains 13.37-15.25% g/g anthocyanosides, 11.23-11.29% g/g magnesium ions (14 mEq), 1.56-1.96% g/g potassium ions; all the results are reported to the dry extract. These results means that the anthacyanosidic extract is a mixture of anthocyanosides and other similar polyphenolic derivatives.

By comparing the IR spectre of the anthocyanosidic extract (fig.1) with the IR spectre of the standard substance, cyanidol chloride (fig.2), a series of bands typical for the chemical groupings in the structure of the substances occurs:

- in the domain 3300-3500 cm^{-1} there is an absorption band because of the vibration of the valence of the hidroxyl grouping in the associated phenols; in this field could also be included an absorption band corresponding to the vibration of valence of the C-O link in the pirilium nucleus;

- the bands corresponding to the vibrations of symmetrical valence of the C-O link are at 1410-1320 cm^{-1} with a peak at 1390 cm^{-1}, whereas the 1120 cm^{-1} band attributed to the variations of asymmetrical valence of the same link can be overlapped on the band caused by the vibrations of valence of the C-C link, which is characteristic for the pirilium nucleus;

- at 1610 cm^{-1} there is an intensive band attributed to the vibration of valence corresponding to the C-C link in the aromatic nucleus, overlapped by the band caused by the ring vibration typical for the pirilium nucleus;

- the band at the 1230-1240 cm^{-1} spectral field can be attributed to the vibrations of the C-H type in the pirilium nucleus;

- the carbonyl grouping in the cynoidic structure can be identified in the IR spectre by the vibrations of valence at 1700 cm^{-1};

- at 1580 cm there is an absorption band determined by the vibrations of the valence of the C=C in the aromatic nucleus;

- at 1350 cm^{-1} and 1180 cm^{-1} there are absorption bands because of the vibrations of the valence of the C-O link in the C-OH grouping;

- characteristic bands for the cyclic structure with heteroatomic oxygen are present at 3200 cm^{-1} and at 1200 v cm^{-1} , at 780 cm^{-1}, 805 cm^{-1} and 850 cm^{-1} ;

- the C-O-C grouping in the structure of the heterocycle with oxygen is highlighted through the band corresponding to the vibration of asymmetrical valence at 1230 cm^{-1} and through the vibration of asymmetrical valence at 1040-1020 cm^{-1}.

In conclusion, in the IR spectre of the anthocyanosidic extract the presence of the phenolic –OH groupings, which are groupings specific to the quinoidic structure, was highlighted when compared to that of the standard

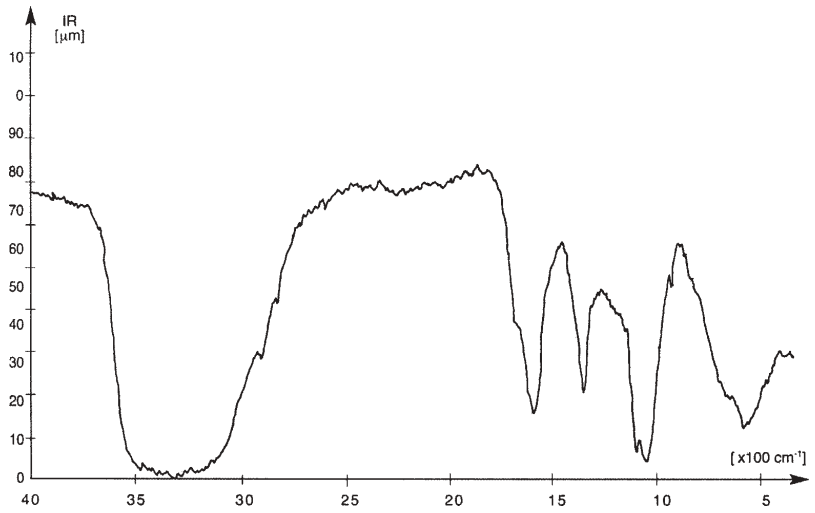

Fig. 3. IR spectre of the anthocyan-magnesium extract

substance. The presence of the vibrations characteristic to the substituted aromatic nucleus was also highlighted.

By comparing the IR spectre of the anthocyan-magnesium complex (fig.3) with the one of the anthocyanosidic extract, it was demonstrated that the anthocyan-magnesium derivative has the same absorption bands with the ones of the anthocyanosidic extract, except for a shift of the band from 3200 cm^{-1}. Bands with a maximum absorption at 1670 cm^{-1}, present in the spectre, were attributed to the vibrations of the C=O valence in the quinoidic molecular structures.

The recording of the spectre in UV-VIS in a solid stage supports what was shown by the IR spectres. The intensity of the bands corresponding to the π–π^* electron transitions at the level of the aromatic systems does not undergo significant changes in the semi-synthesis compound compared to the anthocynosidic extract.

The analysis of all above data shows that:

- the anthocyanosidic extract does not differ structurally from the standard compound, the cyanidol chloride;
- the anthocyanosidic extract coupled with the Mg cation maintains its polyphenolic- type structure; the coupling with this cation is of electrostatic-type.

The behaviour of the analysed compound in the magnetic field led to the following observations:

- the value of the magnetic susceptibility experimentally obtained (158.46 . 10^{-6} cm^3 / mol) is very close to the one resulting from theoretical calculation (172.1 . 10^{-6} cm^3 / mol) and corresponds to a diamagnetic compound, which was to be expected;
- the semi-synthesis compound does not contain paramagnetic species (other metallic compounds or organic radicals).

In future, we have in view to include the anthocyan – magnesium compound in a drug product.

Conclusions

A semi-synthetic compound was obtained by coupling the anthocyans extracted from Ribes nigrum L. (black currant) with magnesium.

The analysis performed with the support of the IR, UV – VIS specters, as well as the measurement of the magnetic susceptibility demonstrated the phenolic-type structure of the semi-synthesis compound and the ionic nature of the anthocyan-magnesium interaction.

References

1.BRUNETON J., Pharmacognosie, Phytochimie, Plantes medicinales, TEC&DOC, Paris, 2005, p. 360

2.GÎRD C.E., Cercetări asupra unor compuºi flavonoidici cu ioni metalici bioactivi în vederea obþinerii de produse terapeutice, Tezã de doctorat, UMF Carol Davila, Bucureºti, 1998

3.GÎRD C.E., BALACI T.D., Antomag comprimate, un nou medicament, Proceedings the 25th Congress of American Romanian Academy of Arts and Sciences (ARA), Cleveland, 2000, p. 231

4.CIULEI I., ISTUDOR V., PALADE M., ALBULESCU D., GÎRD C.E., Analiza farmacognosticã ºi fitochimicã a produselor vegetale, **I**., Ed. Tehnoplast, Bucureºti, 1995, p. 115

5.ISTUDOR V., GIRD C.E., CIULEI I., DUMITRESCU A.M., LUPULEASA D., BALACI T.D., Compozitie farmaceuticã cuprinzând antocian-magneziu ºi procedeu pentru prepararea acestuia, România, nr. brevet 119858 B1, publicat in BOPI nr.5/2005

6.MORAIT GH., Chimie analiticã calitativã, lucrări practice, Litografia IMF, Bucureºti, 1981, p. 125

7.IOVU M., Chimie organicã, Editura Didacticã ºi Pedagogicã, Bucureºti, 1999, p. 82

8.GÎRD C.E., BOSCENCU R., NACEA V., Caracterizarea spectralã a unor noi compuºi terapeutic activi antocian-litiu, Rev. Chim. (Bucureºti), **55**, nr. 1, 2004, p.34

Manuscrip received: 17.12.2007