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DIMERIC PROANTHOCYANIDINS: STRUCTURE AND BIOLOGICAL ACTIVITY

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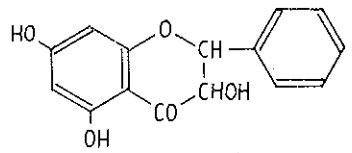
SVMMARIVM — Auctores ostendunt proprietates proanthocyanidinarum dimoerarum, quae ex binis catechinarum unitatibus constant (catechinae sunt anthocyanidinarum praecursatrices). Proanthocyanidinae ex compluribus plantis extrahuntur; earum autem structura determinata est per methodos chemico-physicas et per earum reactiones demolitionis. Auctores novas proanthocyanidinas multas describunt, et antimitoticam quarundam actionem illustrant.

Proanthocyanidins are flavans derivatives which by treatment with diluted mineral acids give origin to anthocyanidins, the red and blue pigments of flowers.

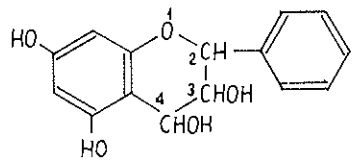
The chemistry of proanthocyanidins has been fully developped only in the last ten years and made possible both by the use of new an efficient preparative methods of separation, mainly chromatography and by the modern physico-chemical approach for the determination of the structures, like high resolution nmr and mass spectroscopy.

Paper presented on April 13th, 1972 during the Plenary Session of the Pontifical Academy of Sciences.

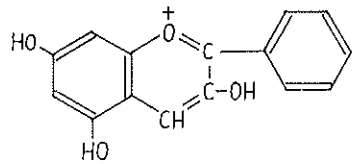
Proanthocyanidins are monomeric and dimeric. Monomeric proanthocyanidins or 3,4 flavan-diols, known also as leucoanthocyanidins, are costituted by one C-15 unit. They represent one stage of oxidation of the flavan system and they may be derived from flavonols.



Flavanonol



Leucoanthocyanidin



Anthocyanidin

Dimeric proanthocyanidins, are formed by two flavan units linked between C-4 and C-8 and thus constituted of 30 carbon atoms.

Since their first discovery by WEINGES [1] in 1961, a number of dimeric proanthocyanidins were isolated and characterized.

The interest of these substances is not only related to their function in the intermediate metabolism of tannins in plants and to the biogenesis of catechins, but also to their inhibiting activity of mitosis [2].

For these reasons we consider interesting to report here the present knowledge of this group of substances on the basis of the results both from litterature and from the researches we have developped in this field since 1967.

The dimeric proanthocyanidins may be classified in regard to the anthocyanidin they give origin: we shall thus have procyanidins, propelargonidins, prodelfinidins, etc.

On the other hand we must consider how the two half moiety of the molecule are linked together, being formed by condensation of two catechin units.

In this case we may have dimeric proanthocyanidins with O:H ratio of n:2n, the so called proanthocyanidins A and by those with the O:H ratio of n:2n + 2, that is, the proanthocyanidins B.

Proanthocyanidins A are less frequently found in nature than those of the group B, and were isolated from *Cola acuminata, Aesculus hippocastanus* and *Vaccinium vitis idea*; their raw formula is $C_{30}H_{24}O_{12}$.

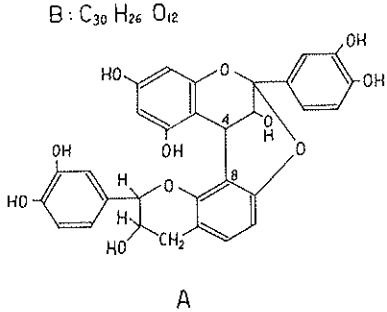
Their structure is not yet completely clarified. According to WEINGES [3] the C-4 of the upper molecule is directly linked to the C-8 of the lower molecule; a second linkage, formed by an ethereal bridge, is present between the two halves molecules.

More frequently are found in plants the proanthocyanidins

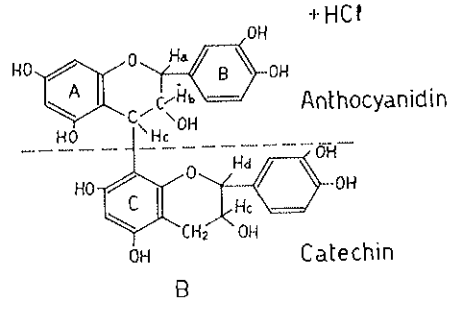
of group B. They are constituted by two hemimolecules not necessarily symmetric linked together by a C-4 C-8 bond.

A : $C_{30}H_{24}O_{12}$

B : $C_{30}H_{26}O_{12}$



A



B

Physicochemical considerations, reactivity of the system and the synthesis are in accordance with this structure [4].

In the case of procyanidins, i.e. of proanthocyanidins which by hydrolysis give cyanidin and catechin, there are 5 centers of chirality and thus 32 possible isomers.

The possibility of existence of these stereoisomers were [5] limited by the fact that the configurations of natural catechins, from which procyanidins probably are formed by condensation and dehydrogenation, are only those of (+) catechin and (—) epicatechin, the only two forms of catechins sofar found in nature.

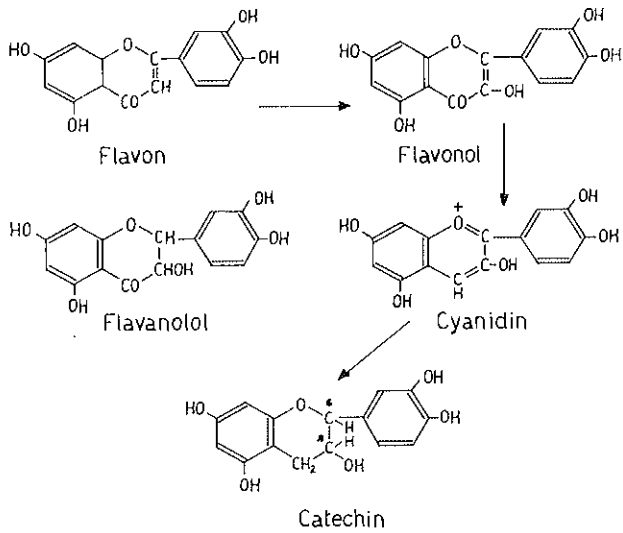
The four possible products resulting from the combinations of these configurations were in effect isolated in plants and named Procyanidin B_1, B_2, B_3 and B_4 (Table I).

The absolute configuration of these procyanidins can be established both from cleavage experiments and by the measure of the coupling constants of the protons on the asymmetry centers.

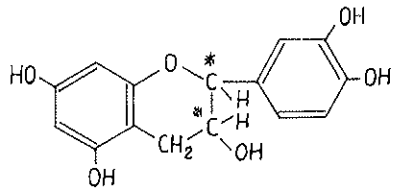
In order to interpretate the structure of dimeric proanthocyanidins it is important to consider before the stereochemistry of catechins.

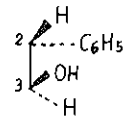
TABLE I — *Dimeric procyanidins.*

	$C_{30}H_{26}O_{12}$		
B_1	(—) Epicatechin	(+) Catechin	*Cola acuminata, Prosopanche americana, etc.*
B_2	(—) Epicatechin	(—) Epicatechin	
B_3	(+) Catechin	(+) Catechin	
B_4	(+) Catechin	(—) Epicatechin	
C	(+) Epicatechin	(+) Epicatechin	*Chamaerops humilis* and other *Palmae*
D	(+) Epicatechin	(+) Epicatechin	
	$C_{30}H_{26}O_{11}$		
	(+) Catechin	(—) Epiafzelechin	*Winstaria chinensis*

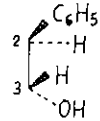


The catechins are 3-flavanols with two asymmetry centers at C-2 and C-3.

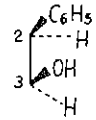




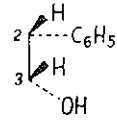
(+ Catechin)



(− Catechin)



(+ epi-catechin)



(− epi-catechin)

Four stereoisomers are therefore possible i.e. (+) catechin (—) catechin, (+) epicatechin and (—) epicatechin.

Till the last year on the basis of the study of a great number of plants it was believed that only (+) catechin and (—) epicatechin were present in nature, beeing the others, found some times in minute quantities, artifacts formed during the extraction by the epimerisation of the formers.

DELLE MONACHE, FERRARI and MARINI BETTOLO [6] have recently and unambigously demonstrated that (+) epicatechin may be found in high concentration, in respect to other catechins in *Chaemaereps humilis* (Palmae) (0,6/kg. respect to 0,1 g/kg.); moreover experimental conditions are such that no epimerisation can take place.

As a further confirmation of the first finding in nature of (+) epicatechin, is the fact that in the same plant is present a new procyanidin, type B, which is formed, as we have demonstrated on the basis of the coupling constants of the nmr spectrum, and cleavage experiments, by the union of two molecules of (+) epicatechin.

On the basis of this finding we may argue that other procyanidins may exist derived not only from the union of (+) catechin and (—) epicatechin but also from the combination of the other two stereoisomers which were before considered not be present in nature.

The above reported considerations on catechin and epicatechin can be extended to all natural substituted catechins.

Among these are known to occurr in nature (+) afzelechin and (—) epiafzelechin, (+) gallocatechin and (—) epigallocatechin, (+) fisidanol (—) robinetidinol and (—) epigallocatechin-4'-methyl-ether.

Among these catechins it was never found one with the (+) epi configuration, before the finding of (+) epicatechin in *Chamaerops humilis* [6]. This results were successively confirmed by DELLE MONACHE, FERRARI and MARINI-BETTOLO [7] studying various plants of the *Palmae* families were it was possible to establish the presence of this stereoisomer. This was also, confirmed by the fact that also (+) epi-afzelechin was found in *Livinstonia chinensis*.

New proanthocyanidins, probably of the B type, were found also in these plants, so that we may argue that several other procyanidins of type B may be obtained from natural sources, resulting from the symmetrical and also asymmetrical combinations of catechins end epicatechins.

The finding in *Ouratea sp.* of a proanthocyanidin which yields by treatment with diluted mineral acids pelargonidin and a new catechin (—) epigallocatechin-4'-methyl-ether indicates the existence of asymmetric dimeric proanthocyanidins [8].

Moreover during the studies on this proanthocyanidins it was found that, both from *Ouratea* and *Chamaerops*, two proanthocyanidins differring only for the configuration at C-4 may be isolated. This was proved because both stereoisomers yield by cleavage the same products.

In Table II we report the proanthocyanidins of type B so far found in plants.

TABLE II — *Dimeric proanthocyanidins.*

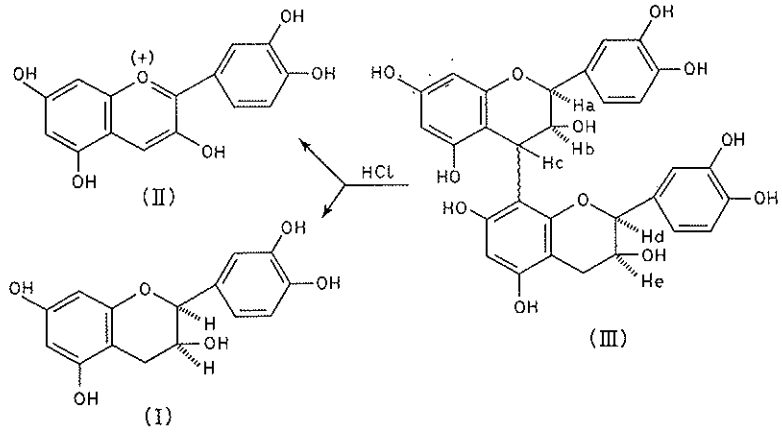
$C_{30}H_{28}O_{12}$	(—) Epiafzelechin	(—) Epi-4'-Methoxy-gallocatechin	*Ouratea sp.*
$C_{30}H_{26}O_{11}$	(+) Fisidenol	(+) Catechin	
$C_{30}H_{26}O_{12}$	(—) Robidenol	(+) Catechin	*Acacia Mearnsii*
$C_{30}H_{26}O_{13}$	(—) Robidenol	(+) Gallocatechin	
$C_{30}H_{26}O_{13}$	(+) Gallocatechin	(+) Catechin	*Myrica Nagi*
$C_{30}H_{26}O_{14}$	(+) Gallocatechin	(+) Gallocatechin	

On a biogenetical basis dimeric proanthocyanidins may be considered formed by the condensation of the enzymatic dehydrogenation products of two catechin molecules or by the condensation of one molecule of leucoanthocyanidin with one of catechin.

The finding in a single plant of the catechins, which constitutes the proanthocyanidin present in the same plant, and the absence of the corresponding leucoanthocyanidins, is in favour of the first hypothesis.

The structure of dimeric proanthocyanidins can be established, as above reported on the basis of their chemical behaviour.

By hydrolysis with diluted HCl of the proanthocyanidin the anthocyanidin chloride and the catechin are obtained. The anthocyanidin corresponds to the upper part of the molecule and the catechin to the lower [10].



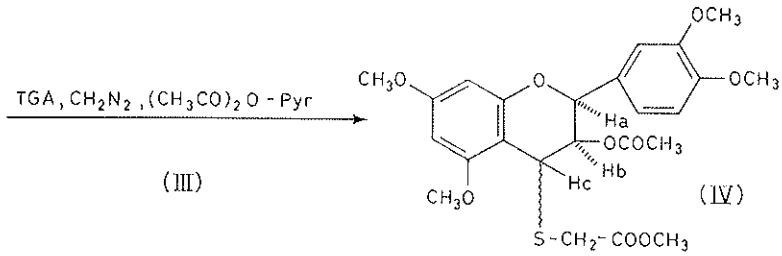
Another very usefull method to determine the structure of proanthocyanidins is the cleavage with thioglycollic acid followed by methylation.

In these conditions the thioglycollate of the flavan, corresponding to the upper part of the molecule is obtained, and the corresponding catechin.

The advantage of this type of cleavage is that the flavan has the same stereochemistry ot C-2 and C-3 as the upper part of the molecule, whereas the acid cleavage does not give any information on this point because of the suppression of the asymmetry centers.

The configuration of the various asymmetry centers can be also deduced on the basis of the coupling constants in nmr spectroscopy of the protons 2, 3, 2′, 3″ (III). More difficult is the interpretation of coupling constants of the proton at C-4.

Also the products obtained in the cleavage experiments can offer informations trough their nmr spectrum about the stereochemistry of the parent proanthocyanidin, mainly the thioglycollate of the flavans.



In the previous cases the spin decoupling experiments in the high resolution spectrometry are very usefull in order to establish the absolute configuration of these products.

A considerable help is also given by the use of MS spectrometry, trough the interpretation of the ion fragmentation which enables us to correlate trough the ions formed various dimeric proanthocyanidins.

* * *

The interest for this new group of natural substances is not only due to their role in the biogenetic pattern of the secondary metabolism of many plants, but also to their effect on tumor growth as reported by various Authors [2, 11].

The first report indicate a general antitumoral effect of some tannins [2]; recently OLIVEIRA et al. have performed a number of experiments with different catechins, tannin and dimeric proanthocyanidins on solid tumors in vivo [11].

The results indicate that catechin are inactive whereas the dimeric proanthocyanidins and in particular « *Ouratea* proanthocyanidin » give significant inhibition of the growth

and even a reduction of solid tumors in vivo (WALKER sarcoma 258).

More complicate molecules of the same flavan type, like the so called Polymer C of *Persea gratissima,* shows very promising activity in the same test.

It is not easy the interpretation of the antitumoral activity of a substance, whose active group are phenolic hydroxyls. OLIVEIRA et al. [11] suggest that the antitumor activity of these substances depends from their affinity for proteins and from a mecchanism similar to that of the tanning of leather with phenolic substances. This explanation altough largely based on well demonstrated reactions does not clarifie the specificity of the action between normal and cancerous cells.

In the last years a number of substances, mainly antibiotics have been studied in relation to their hability in blocking proteic synthesis at various levels.

For actinomycin the mecchanism is explained by a specific interference of this molecule with the double helix of DNA, were the active molecule can intercalate [12].

In order to produce the same effect, in the case of dimeric proanthocyanidins, two factors should be present. First the size of the molecule and the relative distances between active groups should be in range, which would enable the molecule to intercalate in the DNA; secondly the molecule must bear active groups which can react with the DNA basis.

Conformation of the molecule must be also taken in account in order to explain the interaction of the groups.

Several mechanism have been proposed to interpretate the antitumoral activity of synthetic and natural drugs.

Many substances can be considered alkylating agents: in this case is possible that drug interact with DNA, forming twin strands by reaction with 7-N of two adiacent guanine molecules.

Antimetabolites, like fluorouracil and methotrexate interfere with the formation of the DNA.

No explanation was so fare given to explain the acitivity of vinblastin and vincristin, the dimeric alkaloids of *Vinca*.

Proanthocyanidins may be considered rather related, to the latter not for their functional groups, but for their dimensions and shape.

The model and conformational analysis indicates for proanthocyanidins a flat conformation of the moiety of the molecule and thus the possibility to fit in DNA between two basis as reported for acridine molecule by LERMAN [13].

The distance between the two phenyl groups in one of the possible conformations of proanthocyanidins, about 3.4-3.6 A is in accordance with the distance between two basis in the helix [14].

Physico-chemical and biological experiments are in course with the aim of establishing the mechanism of action of these and other dimeric molecules in the processes of mitosis.

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