ALOE TODAY

Part Three

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THE ANTHRANOIDS AND CHROMONE CONSTITUENTS

1. THE DRIED LATEX

As already recalled in Part One (Natural 1, September 2002), Pharmacopoeias do not refer to a drug consisting of aloe leaf but of its dried latex (see Part Two, point 2.1 in Natural 1, October 2002).
In this regard, the American Pharmacopoeia (USP 25, p.) states: "Aloe is the dried juice of the leaves of *Aloe barbadensis* Miller, commercially known as aloe Curaçao, or *Aloe ferox* Miller and its hybrids with *Aloe africana* Miller and *Aloe spicata* Baker, commercially known as Cape aloe …Aloe produces no less than 50% of hydrosoluble extract". It then adds a first experimental method (Assay) to determine this hydrosoluble fraction, and a second method (Alcohol-insoluble substances) to quantize the fraction soluble in alcohol which must not exceed 10% of the initial weight.
The USP does not mention the hydroxyanthracenic derivatives (anthranoids) contained in the leaves or their percentage in the latex; however, they are mentioned by the European Pharmacopoeia 4 (pag. 607) as follows. "Aloe barbadensis. Definition: Barbados aloe is made up of the concentrated and dried latex of the leaves of *Aloe barbadensis* Miller. It contains not less than 28%, referred to the dry extract, of hydroxyanthracenic derivatives, expressed as barbaloin.
Characteristics: masses of a dark brown colour, lightly reflecting or opaque, with conchoidal fractures, or a brown powder soluble in hot alcohol, partially soluble in boiling water, practically insoluble in ether.
Aloe capensis. Definition. Cape aloe is made up of the concentrated and dried latex of the leaves of various species of *Aloe*, especially *Aloe ferox* Miller and its hybrids. It contains not less than 18%, referred to the dry extract, of hydroxyanthracenic derivatives expressed as barbaloin…
Characteristics: masses of a dark brown colour with greenish highlights, with reflecting conchoidal fracture, or a greenish-brown powder soluble in hot alcohol, partially soluble in boiling water, practically insoluble in ether".

1.1 THE ANTHRANOIDS OF ALOE LATEX

Fig. 1 represents some chemical structures of the aforementioned hydroxyanthracenic derivatives, present in the fresh or dried latex of aloe. In this regard, the following observations must be made:
1) I C-glucosides (1) aloin A and aloin B are carbon 10 spatial isomers (stereoisomers), due to the opposing positions of the atom of hydrogen and glucose (the dotted bonding line indicates the direction behind the surface of the page, whilst the unbroken line continues the direction towards who is looking at it, i.e. above the surface of the page). See better below, under point : "Barbaloin".
2) 5-hydroxyaloin A and the carbon 11 rhamnosides of aloin, called aloinosides A and B, are characteristic of Cape aloe whilst le 7-hydroxyaloin A and B characterize Curaçao aloe
3) the anthraquinonic aglycone of aloin is aloe-emodin, present in the latex at minimum concentrations following hydrolytic oxidative processes; the mixture must

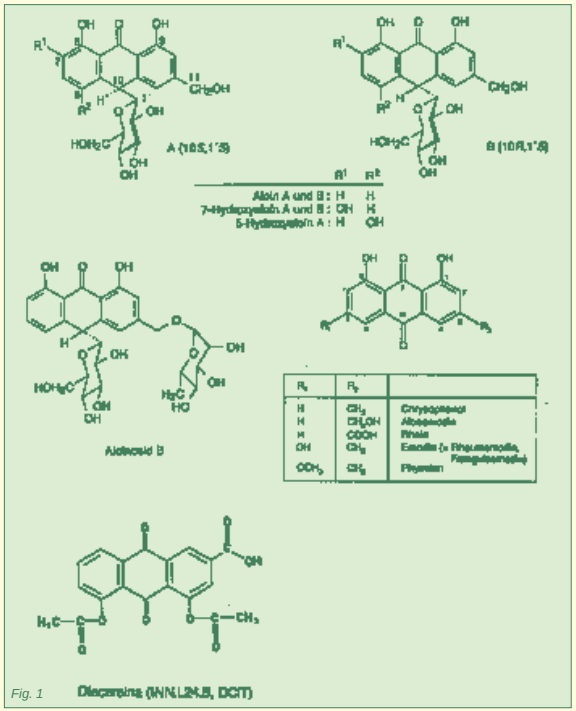

Fig. 1

however also contain traces of other anthraquinonic aglycones such as rhein and chrysophanol (see Natural 1, Part Two, Fig. 2, October 2002 and below) which are formed by hydrolysis of the respective glycosides. As will be said below, there remains the suspicion that the aforementioned aglycones (and in particular aloe-emodin), at certain concentrations, can have a mutagenic activity.

1.2 ALOE RESIN

Alongside the anthranoids, the latex of aloe contains some derivatives of chromones (see Fig. 2 for the chemical structure) which make up the "resin". These are esters with cumaric or cinnamic acid of glucosylchromones (1) called "aloesins" or "aloeresins" with the letters from A to F. Bitter substances ("aloenins" with a chromonic structure (see Fig. 2) have also been extracted from Cape aloe (but not from Curaçao aloe).
In the phytotherapeutic preparations obtained from aloe latex, attempts are made to avoid the presence of aglycones (for the reasons mentioned above) and also of resins and bitter substances because these are deemed to cause unwanted effects which at times accompany the laxative activity of anthranoids (abdominal pain). To this end, the insolubility in water of aglycones, resins and amaroids is exploited (see below under point 2: "The aqueous extract of latex").

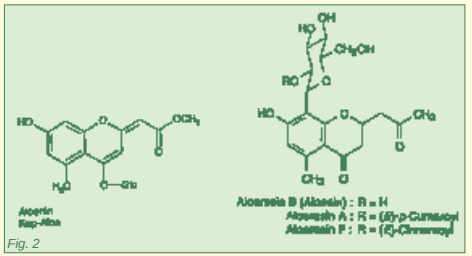

Fig. 2

Due to its pleasantly bitter flavour, aloe latex is however used, in low concentrations, in the alcohol industry (fernet, china, bitter) as an aperitif and as a stomachic: it is well known that the "amari" (Italian "bitters", after dinner liqueurs), by stimulating the lingual papilla, provoke a reflex capable of increasing saliva and gastric secretion (see, in Part Four, the activity of anthranoids).

2. THE AQUEOUS EXTRACT OF LATEX

The concentrated and dried extract of aloe (dried latex, USP 25) is not usually used as such as a laxative, due to the undesired side effects linked with the presence of resins, bitter substances and aglycones. The aqueous extract is usually used which, due to the insolubility in water of these substances, contains barbaloin and its hydroxyanthracenic derivatives, extracted selectively from the water that does not dissolve the resins, amaroids and aglycones.
European Pharmacopoeia reports, on page 609, an "Aloes dry extract, standardized" or "Aloes extractum siccum normatum", but does not offer a method for its preparation. It is simply defined as follows: "The dried standardized extract of aloe is prepared from Barbados or Cape aloe, or from a mixture of the two, by extraction with boiling water. If necessary, it is brought to a content of hydroxyanthracenic derivatives not less than 19% and not more than 21%, expressed as barbaloin calculated with respect to the latex. Characteristics: brown or brown-yellowish powder, scarcely soluble in boiling water." This is followed by a spectrophotometric method to calculate its barbaloin content.
Italian Pharmacopoeia is more explicit (FU IX) which in the monograph: "Dry aloe extract" describes as follows the : "Preparation.
Roughly pulverized aloe p. 1
Water as necessary
One part of the drug is subjected to centrifugation for 10 minutes with 10 p. of boiling water. It is left to rest for four hours and decanted. Five parts of water (referred to the drug) are added to the residue and heated with centrifugation until fallout for 10 minutes; after resting for five hours it is decanted and the liquid is added to the previous one. The collected extracts are filtered and concentrated at reduced pressure at a temperature under 50°C until it reaches a pasty consistency. The extract obtained is dried until a powder or easily pulverized mass is obtained.
Characteristics. Powder or pulverizable mass of a colour variable between brown and black, with a characteristic odour and bitter taste".
The daily maximum posology advised by Commission E (3) for the use of aloe as a laxative is equal to 20-30 mg of hydroxyanthracenes calculated as anhydrous aloin. On the basis of the contents of 20% of aloin in the aqueous extract of latex (Eur. Ph. 4), the posology of the latter would correspond to 100-150 mg/a day. Commission E underlines that the pharmaceutical form of administration should also allow taking lower doses than those stated.

3. BARBALOIN AND ALOIN A / B

3.1 Nomenclature and characteristics

The principal glucoside usually isolated from aloe latex and called "aloin" or also "barbaloin" (from Barbados) is revealed to be not a unitary substance but a mixture of two spatial isomers (see point 1.1) which took the name of aloin A and aloin B, which differ due to the steric position of sugar which, in A is positioned in the direction of the person looking and, in B, in the opposite direction (see Fig. 1). The name of barbaloin therefore (and also simply aloin) indicates a mixture of two substances with an equal weight. According to research carried out at the University of Ratisbon on *Aloe arborescens* (4), the compound produced initially by the plant. in the vegetative state, is only aloin B whilst aloin A is formed, in unforeseeable quantities as it depends on imponderable conditions (perhaps enzymatic) from B (by inversion of the position of the carbon 10 sugar) up to a maximum of 50%, i.e. a ratio of 1:1 of the two isomers, corresponding to the habitual composition of commercial barbaloin. In other words, the young leaves only contain aloin B and are increasingly enriched with aloin A (to the detriment of B) during growth. As the biological reactions take place through steric (spatial) bonds, in which only the groups positioned in the expected direction succeed in bonding with the receptor, we could be justified in asking which of the two aloins can be considered the therapeutic active ingredient. The mechanism of the laxative action of the anthranoid glycosides therefore accounts for their hydrolysis (see below, in Part Four) and the reduction of aglycones and anthrones which would thus be responsible for the activity, independently of the glycoside from which they derive; nor does it appear that literature has highlighted any differences in the laxative power of aloin A and aloin B.
The two compounds differ due to the physical constants, as follows (5) :
aloin A: melting point = 147-148°C

$[a]_D^{20}$ = + 10,2° (in methanol)

aloin B: melting point = 138-140°C

$[a]_D^{20}$ = - 73,0° (in methanol)

and can be separated using the HPLC method, illustrated in Fig. 3 (Hibar Lichrosorb RP18 column, 250 x 4 mm, with mobile methanol-water phase 45+55, revelation at 360 nm) .
The British Pharmacopoeia had, from 1982 to 1988 (BP 88, page 24: "Aloin") a monograph on aloin, describing some of its general and aspecific characteristics; this monograph no longer appeared in the subsequent editions of the BP.
Lastly, it must be considered that where the two forms A and B of aloin are not explicitly mentioned, literature uses the names "aloin" and "barbaloin" indifferently to indicate the aspecific mixture of the two.

3.2 Extraction of barbaloin and of aloin from aloe latex

20 g of pulverized Cape aloe (180) are treated in a 500ml Erlenmeyer flask with 200 ml of water and heated in a boiling bain-marie for 10 minutes under centrifugation. After cooling, it is filtered on to paper in a vacuum and the filtrate is extracted for 6 times with 150 ml each of

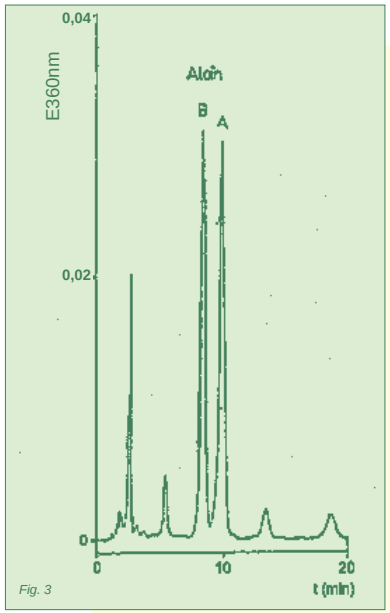

Fig. 3

ethyl acetate (perform TLC of the individual extractions and of the aqueous extract). The ethyl acetate extracts are dried in a rotavapor at 40-60°C, in a 500 ml volumetric flask. The residue (about 3.5 grams) is divided into two portions of equivalent weight. The first portion is crystallized by acetone and, subsequently, by an aqueous solution of one percent of ascorbic acid obtaining approximately 600 milligrams of barbaloin.
The second fraction (5) is taken with a mixture 6+1 of chloroform-methanol, sufficient to obtain a limpid solution in a boiling bain-marie; it is then cooled to –20°C. After a few hours the yellow crystals which have separated are filtered on porous septum; they are washed with 5-10 ml of dichloromethane and vacuum dried at 60°C. The yield corresponds to approximately 1 gram. Analysed by HPLC (see Fig. 3; column: Hibar Lichrosorb RP-18, methanol-water 45 + 55; revelation at 360 nm; retention times: aloin B, 8.5 min; aloin A, 10 min), the product consists of aloin A (80%) and aloin B (20%). It is crystallized again by chloroform-methanol 6+1, it is cooled first at ambient temperature and then for one night at + 4°C obtaining yellow needles of a product which is 95% made up of aloin A and 5% of aloin B. Yield: approximately 300 mg.

4. ALOE-EMODINANTHRONE

Aloe-emodinanthrone represents the aglucone of aloin (barbaloin) and is considered the active metabolite to which the laxative action of both the latex and aloin is due (see: Part Four).
It can be prepared from aloin or from aloe-emodin.

4.1 Preparation of aloe-emodinanthrone from aloin

Obtaining anthrone by hydrolysis of aloin takes place due to the glucosylic carbon-carbon bond, in special conditions (reducing atmosphere and exclusion of oxygen) as follows:
20.0 g of aloin are suspended in 500 ml of an aqueous solution of 40.0 g of borax and 4.0 g of ascorbic acid and are kept boiling for 3 hours in nitrogen flow. After cooling, the limpid reddish brown solution is acidified with diluted hydrochloride acid and then placed for 12 hours in a refrigerator. The separated precipitate is filtered, vacuum dried and extracted in Sohxlet with benzene. By cooling the benzene solution, yellow-brown crystals of aloe-emodinanthrone are obtained which are recrystallized by methanol at 20% of acetic acid until red-orange crystals with a melting point of 191-192°C (decomposition) are obtained. Yield 1.25 grams.

4.2 Preparation of aloe-emodinanthrone from aloe-emodin

For aloe-emodin see below, point 5.
20.0 grams of aloe-emodin are dissolved in 200.0 ml of boiling acetic acid and, after the addition of 70.0 ml of a solution at 40% of conc. stannous chloride, it is heated to fall for 3 hours. By cooling the solution, the aloe-emodinanthrone is crystallized in red-orange needles, which were recrystallized by methanol at 20% of acetic acid.
Melting point: 191-193°C, Yield: 950 mg.

5. ALOE-EMODIN

Aloe-emodin (see Fig. 1) is the product of oxidation of the respective anthrone (see point 4) but is also present in traces in aloe latex, both as such and as a glycoside; in the intestine it is reduced to the active form of anthrone. Due to a suspected mutagenic activity, its presence in laxative products should be avoided as a precaution; recent studies show its activity against some tumoral forms (see Part Four).
It can be prepared by oxidation with ferric chloride of the respective anthrone but the most immediate method of preparation appears to be that of oxidative hydrolysis of aloe latex or aloin.

5.1 Preparation of aloe-emodin from aloe latex

100 g of aloe latex in power are suspended in 1.5 litres of water, 500.00 grams of ferric chloride are added and the solution is heated to fall out for 3 hours. After cooling, the precipitate is filtered, washed with water and dried. The blackish residue thus obtained in micronized and extracted in Sohxlet with toluene. From the filtered toluene solution, red orange crystals of aloe-emodin are separated, which is recrystallized by toluene and then by methanol. Yield 1.7 grams; melting point: 221-223°C.

5.2 Preparation of aloe-emodin from aloin

40.0 grams of aloin are suspended in a solution of 200.0 grams of ferric chloride in 600 ml of water and heated until fall out for 5 hours. The blackish precipitate which has separated by cooling, is filtered and, after drying, is pulverized and extracted in Sohxlet with toluene proceeding as stated in point 5.1. Yield 3.6 g of pure product at melting point 220-222°C.

6. RHEIN AND DIACETYLRHEIN

Rhein (see Fig. 1) is a product of oxidation of aloe-emodin, the primary alcoholic group of which, in position 3, is replaced by a carboxyl. It belongs to the group of aloin aglycones but also represents the final product of the biological metabolization of aloe which can be found in maternal milk and urine.
It presents a pharmaceutical interest because its diacetyl derivative (fig. 1), commercially called "diacerein", has anti-arthritis properties and acts against degenerative joint affections. It is possible to transform aloin into diacerein, and/or rhein, as follows:

Preparation of diacetylrhein and rhein from aloin.

10.0 grams of crystallized barbaloin (see above under point 3.2) are heated, under centrifugation, for one hour at 50-60°C in 100 ml of acetic anhydride containing 10.0 grams of anhydrous sodium acetate. After cooling, iced water is added to the solution until precipitation of the acetylaloin. This is filtered, dried and dissolved again in 100ml of acetic anhydride, with the precautionary addition of a solution of 12 grams of chromic anhydride in 55ml of acetic acid and 4ml of water, maintaining the solution under centrifugation, for two hours, at 50-60°C. By cooling, yellow crystals of diacetylrhein (4.5 grams) are separated, which, after crystallization by acetic acid, show a melting point of: 218-219°C.
The precipitate (4.5 grams) is dispersed in 80 ml of aqueous solution at 10% of sodium hydrate, heated in a boiling bain-marie for 30 minutes and then poured into 120 ml of hydrochloric acid at 10%. 2.9 grams of rhein are obtained in the form of yellow flakes which are crystallized twice by pyridine. Yield: 2.1 grams of pure rhein with melting point of 318-319°C.

LITERATURE AND NOTES

(1) If the fraction without sugars of the molecule is bonded to glucose, the compound obtained is a "glucoside"; if it is bonded with other sugars (including with glucose), it is defined a "glycoside" and the parts without sugars will be indicated respectively as "aglucone" or "aglycone". In turn, the aglycone-sugar bond of glycosides is mainly carbon-oxygen-carbon and, more rarely, carbon-carbon. In the latter case, we talk of C-glucosides (C-glycosides) or glucosyls (glycosyls) as in aloins there are 10-glucosyls of aloe-emodin.
Often, more simply, the terms "glycoside" and "aglycone" are used to indicate all the above cases.
(2) As already observed in point 1, "aloe" refers to the "dried latex of the leaves" as defined in the relative USP 25 monograph.
(3) See "Le Monografie Tedesche" under Aloe. Studio Edizioni.
(4) GrünM. and Franz G., "Untersuchungen zur Biosynthese der Aloine in Aloe arborescens", Arch. Pharm. 315 , (1982) 231
(5) Adam K.P. and Becker H., "Analytik biogener Arzneistoffe", WVG, Stuttgart 2000 p. 412