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Naturally Occuring Quinones and their Related Reduction Forms: Analysis and Analytical Methods

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Recent advances in analysis and analytical methods for naturally occuring quinones and their related reduction forms are briefly reviewed with respect to the isolation techniques, the qualitative and quantitative analysis, including pharmacopoeial analytics, and the structure determination. The main thrust is the analytical application to the acetate-derived class of anthranoids, particularly the group of diastereomeric 10-C-glucosylated anthrone and oxanthrone derivatives, the absolute configurations of which are reported here for the first time. The chapter covers literature data from 1982–89 in particular.

Analyse und Analysenmethoden natürlich vorkommender Chinone und ihrer Reduktionsformen

Die vorliegende Übersicht berichtet über neue Entwicklungen auf dem Gebiet der Analyse und Analysenmethoden für natürlich vorkommende Chinone und deren Reduktionsformen: Präparative Isolierungstechniken (Kap. II); qualitative und quantitative Analyse (Kap. III) unter besonderer Berücksichtigung der Arzneibuchanalytik (Kap. IV) sowie Methoden der Strukturanalyse (Kap. V). Schwerpunktmäßig wird die Analytik acetogener Anthranoide, insbesondere die der diastereomeren 10-C-Glucosylanthron- und oxanthronderivate behandelt, deren absolute Konfigurationen hier erstmals veröffentlicht werden. Das Referat berücksichtigt hauptsächlich Literaturangaben des Zeitraumes 1982–1989.

Keywords: Quinones, anthranoids, analytical methods, chromatography, plant products analysis, plant chemistry, pharmacopoeial analytics, spectroscopy, structure determination □

I. Introduction

The purpose of this report is to review current knowledge and recent advances with respect to the isolation and analysis of naturally occuring quinones and their related reduction forms. It includes three sections based on those methods which characterize the essential steps in the analysis of natural compounds: isolation and purification with special consideration of preparative chromatographic techniques (II); qualitative and quantitative analysis by chromatographic, spectroscopic and electrochemical techniques or by chemical determination with special consideration of pharmacopoeial analytics (III/IV); structure elucidation and stereochemical analysis (V).

The chapter covers literature data from 1982 to 1989 in particular, since when about 400 papers dealing with one or more aspects of the chromatography, spectroscopy and/or chemical determination of natural quinones have appeared. Most of these, however, simply tabulate single data on special compounds without presenting any evaluation or processing of these data in analytical or structural respects. Although of high value in a special context, they may be of minor interest in a brief survey like this. Thus, the first 17 references cited mainly quote review articles, books and other monographs, in which the subject matter is either discussed in a greater detail, or for certain quinonoid structural types or groups. The more than 1500 naturally occüring quinones, which are at present known, have been divided formally into six groups, and further subdivided on the basis of structural types into smaller groups (10, 12), e.g. the division "benzoquinones" contains 11 different subdivisions. If one considers the structural variations within each subclass, each of which has its own physical and chemical properties, then it is clear, that one cannot describe newer analytical developments for all quinonoid groups or novel structure types in a comprehensive manner. Rather, recent advances in methodology or new trends within known methods for the analysis of plant quinones are discussed, which are of broader application for particular substance groups or structure types, or those, which, in the author's opinion, are of importance for preparative, applied or mechanistic chemistry and/or biochemistry. The main thrust will be the analytical application to the acetate-derived class of anthranoids particularly to the group of the diastereomeric 10-C-glucosylated anthrones (= aloin- and cascaroside-type) as well as the newly found oxanthrones resp. (= 10-hydroxyaloin- and 10-hydroxycascaroside-type; see fig. 1), the latter of which are introduced here for the first time.

II. Isolation Techniques

1. Extraction and Artifacts

Before discussing the various separation methods, some special features of extraction and purification are described

For Prof. Dr. Georg Schneider, Frankfurt/M., on the occasion of his 65th birthday.

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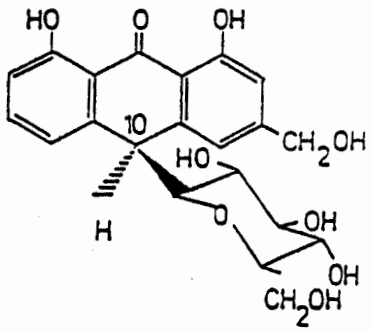
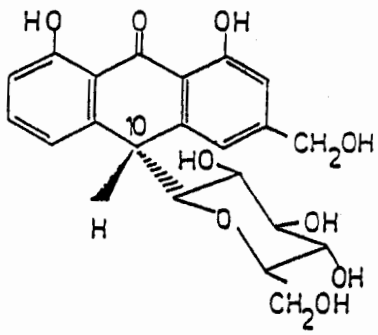
The Generic Structures of the 10-C-Glucosyl-anthrones [8a]:



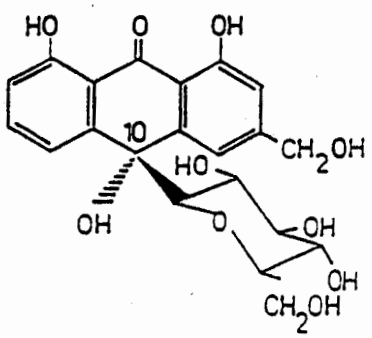
Aloin A (10S, 1'S)



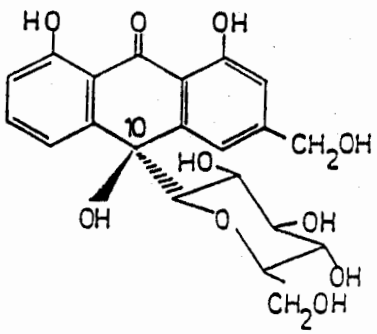
Aloin B (10R, 1'S)

Occurence: Aloe spp., Rhamnus purshianus

The Generic Structures of the 10-C-Glucosyl-oxanthrones [8b]:



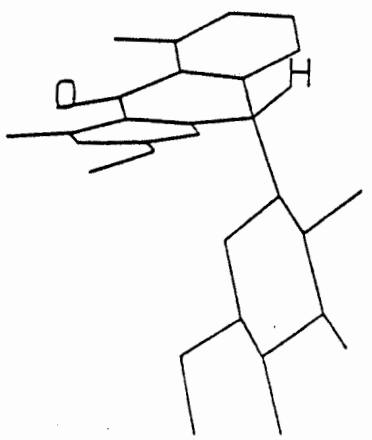
10-Hydroxyaloin A (10R, 1'R)

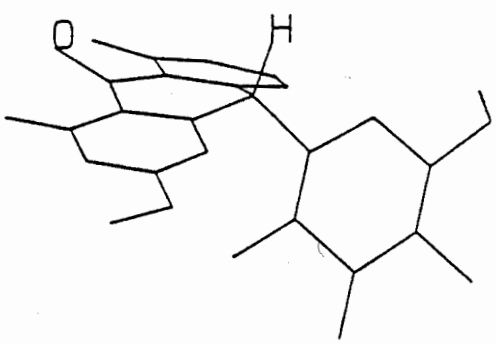


10-Hydroxyaloin B (10S, 1'R)

Occurence: Rhamnus purshianus, but not Aloe spp.

The Conformers of Aloin A: the Extra- und the Intra-Form [8c]





In both anthrone- and oxanthrone-10-C-glucosyls, the anthrone skeleton is not planar due to the sp^3-hybridization at C-10 and two conformations are possible: the extra- and the intra-form. On the left, the extra-conformer of Aloin A is shown: carbonyl oxygen and glucosyl residue are in cis-position. In the intra-conformation (on the right) carbonyl oxygen and glucosyl residue are trans-positioned [8c].

Fig. 1: Structural Formulae of the Aloin- and 10-Hydroxyaloin-types

Extraction of quinones from plant material generally poses no problems, since most quinones are chemically stable. It is difficult to recommend any completely general extraction procedure because natural quinones vary so much in polarity and solubility. For example, many quinones can be taken up in non-polar solvents such as ether and chloroform, whereas glycosides, chiefly of anthranoids, obviously require polar solvents. Original papers should be consulted for details. Some individual procedures should be mentioned: an improved extraction of quinonoid compounds from dried plant material in aqueous media can be accomplished by the pressure expansion (PEX) procedure, in an atmosphere of carbon dioxide (3.5–35 bar for 2.5–10 min), as demonstrated with bianthrones from Senna and Rheum drugs (18), which yielded twice as much of the anthra-derivatives when compared to normally processed material. A sequential extraction of isoprenoid quinones, such as menaquinones and ubiquinones from microorganisms, was developed using a two phase mixture of petroleum ether and methanolic saline solution (19). Isolation of the reduced forms, e.g. anthrones or hydroquinones, from fresh plant material or cell cultures needs special precautions to prevent their oxidation: individual anaerobic procedures (20) (plastoquinones and plastoquinols), the presence of solid carbon dioxide (21), dithionite salt/nitrogen (22), liquid nitrogen (23), avoidance of elevated temperatures and the exclusion of daylight (24). One problem associated with isolation procedures, to which is often not given enough attention, is the formation of artifacts. This may already have taken place during the process of extracting the plant material. Some anthraquinones, which have been isolated, exhibit the presence of 2-methoxymethyl- or 2-ethoxymethyl-groups, which are believed to be artifacts formed during extraction with methanol or ethanol as solvents (16). Juglone-type naphthoquinones may also be converted at room temperature in methanol solution into both 2- and 3-methoxy substituted derivatives (25). Additionally, 7-methyljuglone, the major naphthoquinone of the Juglandaceae and Ebenaceae, tends to undergo conversion reactions when adsorbed on silica gel exposed to air, and is rapidly oxidized to methylnaphthazarin and four dimeric naphthoquinones (26). Diastereomeric 10-C-glucosylanthrones, such as aloins A and B (fig. 1), are unstable in solution, where they are subject to interconversion via the tautomeric anthranol (27, 28). In heated aqueous alcoholic solutions, or optimally in the presence of ammonia at pH 9, the aloins are oxidized to the corresponding oxanthrone derivatives 10-hydroxyaloin A and B (29) (fig. 1), as shown in a structural revision of the published 4-hydroxyaloins (30). In this context, alkaline extraction, sometimes used with anthranoids, is not recommended for quinones with exposed quinonoid positions open to nucleophilic attack (11).

2. Separation Methods

No special methods are required for the isolation and purification of quinones. Although numerous new separation techniques have become commercially available over the last decade, the standard procedures are based on thin layer chromatography (TLC) using silica gel and open column chromatography (CC), in addition, purification is often achieved by crystallization or recrystallization procedures. These techniques still play the major role in the isolation of quinones, but obviously no particular solvent system can be recommended for all quinones. For example, quinone isolation from microorganisms (2) follows a standard practice, therefore original papers should be consulted for details. Several classical and modern chromatographic procedures have been used. These include planar techniques, such as preparative and centrifugal TLC, open CC (silica, Sephadex LH-20, polyamide, cellulose, XAD-2) and preparative pressure liquid chromatography (low, medium, high pressure and flash chromatography). Methods using a counter-current principle are: droplet counter-current-chromatography (DCCC), rotation locular countercurrent-chromatography (RLCC) and centrifugal countercurrent chromatography (CPC). Their applications in the quinonoid isolation are discussed below, without considering chromatographic parameters, advantages, limits and disadvantages (for these considerations see (4)). Finally it should be mentioned that the techniques presented are not mutually exclusive, but complementary to each other. In most cases, pure compounds from crude plant extracts can only be obtained rapidly by combining the various available chromatographic techniques.

As mentioned above, preparative TLC in conjunction with open CC is still to be found in the majority of publications covering the isolation of natural quinones, especially in work from those laboratories without access to modern separation techniques. In overcoming some of the known drawbacks of classical preparative TLC, centrifugal TLC has been proved to be superior in several important aspects (4), however the resolution is limited and cannot be compared with that of HPLC (31). Four applications described with quinones, three of them in artificial mixtures, carried out with the commercially available "Chromatotron" (anthraquinones (32), naphthoquinones (33) and "Rotachrom" (anthraquinones (34, 35)) using sequential CTLC and centrifugal planar CC, indicate, that centrifugal TLC has been employed only for the isolation of aglycones, which are in general easier to separate than quinone glycosides. In this context, to help in optimising the mobile phase, the so called PRISMA model, based on Snyder's solvent selectivity triangle, was employed (36). This model has already been used for classical and over-pressure TLC as well as HPLC. The PRISMA technique is especially useful for ternary and quaternary solvent systems but less so for binary systems, the most commonly used CTLC eluents. As mentioned, quinones are frequently separated on a preparative scale by means of open CC using silica adsorbents. Columns packed with Sephadex LH-20 are also widely used, for example for the fractionation of anthracene derivatives from Aloe (37) and Kniphofia spp. (38), or recently in the separation of closely related diterpenoid quinones of the royleanone-type or plectanthrone-type from Coleus and Salvia species (39).

A further example is the efficient separation of labile glucofrangulinanthrones A and B, which was achieved in combination with MPLC (22). CC on XAD-2, a polystyrene-divinylbenzene copolymer, has been used for the purification of anthranoids from Rhamnus, Rheum (40) and Rubia spp. (41). Some applications using supercritical fluid chromatographic (SFC) techniques for the isolation of commercial dyes, e.g. synthetic anthraquinones are discussed in (13). According to ref. (4) the term "pressure liquid chromatography" is used here to include any method involving the application of pressure to a chromatography column, as distinct from gravity-driven CC separations. Thus, all categories ranging from flash LC with 2 bar to preparative HPLC with 100 bar, and sample sizes from µg to g can be included under this heading.

The simple technique of flash chromatography, basically an air pressure-driven hybrid of medium pressure and short column

chromatography, allows particularly rapid and efficient separations, as is demonstrated by the isolation of hydroquinone diterpenoids from Clerodendrum spp. (42) or naphthoquinones from Diospyros usambariensis (33) or anthracene derivatives from Psorospermum sp. (97).

Only a few applications are described using a low pressure LC system: a series of diterpenoid quinones, among these royleanone from Coleus carnosis has been isolated by chromatography on Lobar Si 60 columns with various hexane-acetone mixtures (43). The MPLC technique involves the use of longer columns with large internal diameters and requires higher pressures than LPLC (5–20 bar) to enable sufficiently high flow rates. MPLC, using RP-18 silica gel and a solvent system containing MeOH-H_2O-HCOOH, in combination with Sephadex LH-20 open CC was employed for the preparative scale separation of labile glucofrangulinanthrones A and B, the closely related primary bisglycosides of fresh Rhamnus frangula bark (22). The complete separation takes no longer than 3.5 h, of which only 15 min. is required for the MPLC.

As for natural product isolation, preparative HPLC is still rarely used within quinones: one application of preparative HPLC by the radial compression system of WATERS PrePAK 500, using RP mode on octadecylsilyl bonded columns, is described for anthraquinone antitumour agents from fermentation broths of Actionsporangium sp. (44) (sample sizes: 3–25 g; mobile phase: CH_2Cl_2-MeOH-H_2O-NH_4OH). The presence of ammonia in the eluting solvent led to problems such as deactivation of the columns and a shortening of column life. Furthermore, tetraprenylated toluquinones and -quinols were obtained from the brown alga Cystoseira jabukae (45) by preparative LC on a Jobin-Yvon Miniprep chromatograph (LiChroprep Si 60; C_6H_{14}-Et_2O 9:1 and 6:4).

All the techniques mentioned so far, use a solid stationary phase or a liquid stationary phase fixed on an "inert" support. In order to avoid the particular complications arising from solid supports, various support-free counter-current chromatographic techniques based on liquid-liquid partition have been developed in the last decade (4). Among these DCCC has established itself as a standard laboratory procedure for the separation of natural products. DCCC also has been successfully applied to the isolation of quinonoids, particularly of various anthracene and napthalene derivatives. 21 applications are summarized in table I. In our laboratory, as the method of choice, DCCC is used not only for separating the 10-glucosyl-anthrones and -oxanthrones from Aloes and Cascara, but also for resolving each of them into their two stereoisomers A and B, which produced altogether 21 new compounds within this class. The fundamental experiment was the preparative scale separation of the aloins A and B (7) (fig. 1), which could formerly only be accomphished by HPLC (27, 28) or by fractional crystallizations (27). The solvent composition was governed particularly by consideration of the partition coefficients of both diastereomers, using HPLC to determine the partition of aloins A and B between the two solvent phases (aloin A: K=2.18; aloin B: K=2.53). Despite the conversion of aloin A into aloin B in aqueous solutions, the DCCC method is sufficiently rapid to avoid this problem. Compared to DCCC, the rotation locular or centrifugal counter-current chromatographic techniques have found only very restricted application in the area of quinonoids. Various chromomycins, clinically used antitumour antibiotics from the crude chromomycin extract of Streptomyces griseus culture broths have been successfully separated by RLCC using solvent systems based on $CHCl_3$/EtOAc/MeOH/H_2O (46). Lipophilic plectanthrone-type quinones from Plectranthus sp. were enriched by RLCC using the system n-hexane/Et_2O/PrOH/EtOH/H_2O (47). A method-critical comparison of RLCC to DCCC was shown for the separation of aloins (48): though the sample sizes were only one fifth of the amount of aloins (60 mg), the resolution was low, aloin B could not be separated. Centrifugal liquid-liquid partition chromatography (CPC) with a Sanki CPC apparatus has been used to perform rapid separations of anthranoid pigments from Psorospermum febrifugum and naphthoquinones from Diospyros usambarensis (49). The technique of horizontal flow-through coil planet centrifuge (CHFCPC) was applied for the separation of some antitumour agents of lipophilic daunorubicin-type anthraquinones and their metabolites (50, 51) and for the isolation of the diaziridinyl-benzoquinone diaziquone and its hydrolysis products (52).

III. Qualitative and quantitative Analysis

In this section the various chromatographic and, in shortened form, electrochemical methods used for detection and determination of quinones are discussed. Recent developments in analytics of officinal pharmacopoeial anthranoid drugs or of their anthranoid constituents are considered in Ch. IV. Individual applications in the quantitative determination of certain quinonoid compounds or types using special physical methods (mainly spectroscopy and polarography) or chemical methods (mainly colorimetric determinations) may be taken from review articles (e.g. 1, 10, 11, 12, 53).

A very important step in obtaining correct results is the correct sample preparation, particularly when dealing with biological material. In addition to liquid-liquid partitioning extraction techniques, liquid-solid or solid-phase (SPE) extraction has in recent years proved to be a very useful technique, because it offers faster and more selective sample preparations than liquid-liquid techniques. In our laboratory (54) SPE was used for the selective fractionation of the aloins from an Aloe extract, which has been formulated in a phytopharmaceutical, with five other plant extracts in dragee form. By means of SPE on a C_{18} silica extraction column the aloins could be separated from further polyphenols and other constituents by conditioning with MeOH/H_2O (2:8) and elution with MeOH/H_2O (8:2).

As with other organic molecules, quinones may be detected and determined by chromatographic methods. Since most quinones are coloured, they may be easily detected in TLC or PC even with the naked eye. Together, TLC and HPLC techniques play the major role in the qualitative and quantitative analysis of plant quinones. TLC on silica, by far the most popular adsorbent, is recommended as an inexpensive, simple, fast and effective method for the qualitative characterization of quinones: it should be stated however, that some quinones are highly unstable in the adsorbed state, especially when exposed to light, hence there may be "tailing" due to the formation of hydroquinones, which, in turn, prove to be partially air oxidized, when subjected to TLC (cp. Ch. II). TLC is indispensable for the checking of a crude plant extract for quinones, particularly within the scope of pharmacopoeial analytics (see Ch. IV), and often provides some structural information (1, 10, 11, 13). TLC is also very useful in monitoring fractions obtained in the preparative separation by other methods and in testing homogeneity. Furthermore, TLC is of great help in the

selection of solvents for HPLC and other pressure CC techniques as well as for the above-mentioned counter-current chromatographic techniques. As already recommended for the preparative separation methods in Ch. II, original papers or review articles on certain quinonoid structural types (e. g. prenylquinones (2. 5), perylene quinones (55), pluramycin (56) or anthracycline (57) antibiotics) should be consulted for details of TLC analysis, especially for typical colour reactions. Here, consideration of TLC is restricted to the analysis of pharmaceutically interesting emodin-type anthranoids, as discussed in Ch. IV.

Analytical HPLC is now a well established, powerful and predominant technique useful for the separation of numerous types of plant quinones. HPLC has mainly been employed for quantitative determination, purity verification of isolated compounds or for chemotaxonomical comparison of quinonoid compounds and will in the near future certainly

Table 1: Quinonoids and their Reduction Forms isolated by DCCC

Quinonoids	Solvent system	Plant source	Reference
Rubiaceous anthraquinones			
Lucidin primeveroside	$CHCl_3$–MeOH–H_2O (5:5:3)	Gallium mollugo	Inoue et al. (1979)
Aglycones and glycosides	EtOAc–n–PrOH–H_2O (7:3:9)	Morinda citrifolia	Inoue et al. (1981)
Ruberythric acid	n–Hexane–EtOH–H_2O–EtOAc (5:4:1:2)	Rubia cordifolia	Hokawa et al. (1983)
Rhamnaceous anthraquinones/ naphthalides			
Sorinin, 6-methoxysorinin and their monoglucosides	n–BuOH–MeOH–H_2O (5:1:5)	Rhamnus catharticus	Rauwald, Just (1983) (107)
Emodinprimeveroside/-gentiobioside/ -glucoside	$CHCl_3$–MeOH–H_2O–HCOOH (7:13:8:0.1)	Rhamnus catharticus	Rauwald (1983)
Alaternin, 2-methoxystypandrone (aglycones)	n-Hexane-MeOH (non aqueous) (5:1)	Rhamnus fallax	Miething, Rauwald (1987)
Rhamnalpinoside and its aglycone	$CHCl_3$–MeOH–H_2O–HCOOH (7:13:8:0.1)	Rhamnus fallax	Miething, Rauwald (1984) (104)
Bianthrone glycosides			
Sennosides A/B	$CHCl_3$–MeOH–n–PrOH–H_2O (9:12:1:8)	Rheum sp.	Ogihara et al. (1976)
Sennosides A/B	n–BuOH–Me_2CO–H_2O (33:10:50)	Cassia sp.	Hostettmann (1984)
Diastereomeric 10-C-Glucosyl-anthrones and -oxanthrones			
Aloins A/B	$CHCl_3$–MeOH–H_2O (7:13:8)	Aloe sp.	Rauwald (1982)
6'–O–p–Coumaroyl–aloins A/B	$CHCl_3$–MeOH–H_2O (7:13:8)	Aloe barbadensis	Rauwald (1988)
7-Hydroxyaloins A/B	$CHCl_3$–MeOH–i–PrOH–H_2O (10:10:1:8)	Aloe barbadensis	Rauwald (1983)
6'–O–Acetyl–and 4',6'–O–diacetyl–7–hydroxyaloins A/B	$CHCl_3$–MeOH–H_2O (7:13:8)	Aloe succotrina	Rauwald, Diemer (1986) (126)
6'–O–p–Coumaroyl–7–hydroxyaloins A/B	$CHCl_3$–MeOH–H_2O (7:13:8)	Aloe barbadensis	Rauwald, Diemer (1987) (126)
8–O–Methyl–7–hydroxyaloins A/B and 6'–cinnamoylderivatives	$CHCl_3$–MeOH–H_2O (7:13:8)	Aloe barbadensis	Rauwald, Niyonzima (1990)
5–Hydroxyaloin A	$CHCl_3$–MeOH–H_2O (7:13:8)	Aloe capensis	Rauwald (1987) (99)
Homonataloins A/B	$CHCl_3$–MeOH–H_2O (7:13:8)	Aloe lateritia	Rauwald, Niyonzima (1989) (130)
10-Hydroxyaloins A/B	$CHCl_3$–MeOH–H_2O (7:13:8)	Rhamnus purshianus	Rauwald, Lohse (1989)
10-Hydroxycascarosides E/F	BuOH–MeOH–H_2O (5:1:5)	Rhamnus purshianus	Rauwald (1989)

become also more popular as a method for their isolation on a semi-preparative or preparative scale (cp. Ch. II). Regarding the nature of column packing materials, in most of the literature reviewed here, separations on silica gel columns, mostly reversed phase columns (C_{18} or C_8) have been used and only few on amino-chemically bonded silica gel or on the more polar nitrile-chemically bonded phase CN. The recent availability of a photodiode array detector for HPLC has enabled a marked improvement in the possibilities of peak identification. Simultaneous detection at different wavelengths and measurement of the UV spectrum of each separated compound during the elution allow an easy characterization of quinones. An interesting application shows the simultaneous detection and determination of reduced glucofrangulinanthrones (λ_{max} 320nm) and oxidized glucofrangulins (λ_{max} 420nm) A and B from **Rhamnus frangula bark** or commercial frangula drug, which has not correctly been stored for a year (22). In the following, some recent HPLC procedures for the separation and quantitative determination of various selected quinonoid groups, excluding the 1,8-dihydroxyanthranoids (see Ch. IV), are presented in a keyword form to demonstrate the comprehensive use and possibilities of this technique for all groups of quinonoids: Rubiaceous anthraquinones (16), particularly from Rubia spp. (58); insect pigment anthraquinones of the kermesic-acid type (e. g. from Coccus cacti (59)), using the eluent MeOH/H_2O/HCOOH and RP-18 material: reduced tetrahydroanthraquinones from Alternaria porri (60); hydroquinone (61, 62); pigments from fungi (3) (terphenylquinones, benzoquinones etc.); pluramycin antibiotics (56); anthracyclinone antibiotics of the doxorubicin or daunorubicin-type (57) or the new elloramycin type from Streptomyces divaceus (63) (diode array detection; gradient elution with acetonitrile and 0,1% phosphoric acid). 13 abietane-type diterpenoid quinones from Plectanthrus sanguineus have been analysed by non-aqueous cation-exchange HPLC using a Partisil 10-SCX column and gradient elution (39). For the analysis of phytotherapeutically used naphthobianthrone derivatives of the hypericin type from Hypericum perforatum herb an ion-pair chromatographic method with gradient elution (64) (MeOH-EtOAc-NaH_2PO_4/buffer; RP 18) or an RP-18-HPLC method using a water-acetonitrile-methanol gradient (plus phosphoric acid) (65) have been described.

For the group of the non-isoprenoid naphthoquinones, only few HPLC applications have been described: Juglone- and lawsone-type naphthoquinones from a ligroin extract of Diospyros usambarensis have been separated using 1% acetic acid in n-hexane as solvent and a CN bonded phase (66). A safrole stationary phase and MeOH/H_2O as eluent were used to examine the chromatographic behaviour of several naturally occuring naphthoquinones, from Bignoniaceae, e. g. Radermachera sinica (67). For the reduction of lapachol and five related naphthoquinones from Bignoniaceae spp. tandem HPLC methods are available (68), one of which uses a chiral column (covalent leucine; hexane-isopropanol; isocratic and gradient systems) which resolves α-/β-lapachone and analogues well, whereas the quinols lapachol and isolapachol are retained. A naphthoquinone, 2-methoxystypandrone, from Rhamnus fallax was separated together with the characteristic 1,8-dihydroxyanthraquinones (69) (RP18; gradient elution with MeOH/H_2O and MeOH).

Most analytical work on naphthoquinones has been confined to the highly lipid-soluble prenylquinones, the K-vitamins and the prenylbenzoquinone derivatives (2, 5, 9) (ubiquinones, plastoquinones, tocoquinones), which are of particular biochemical interest. Thus, in the last five years over 40 papers have been published, mainly by biochemical working groups (these references may be requested from the author; for reviews see (2, 5, 9)). These isoprenoid naphthoquinones are best resolved by RP-HPLC using aqueous methanol mobile phases (2, 5, 9), while non-isoprenoid naphthoquinones, mentioned above, require a high water content for separation by RP-HPLC with methanol-water systems (66). Additionally to the groups of K-vitamins and 1,4-benzoquinones, a new simple and selective spectrophotometric determination of menadione and other related p-quinones has been developed for the purity test of phytonadione in Japanese Pharmacopoeia using 3-ethylrhodanine (131).

In the HPLC procedures so far described, quinones are determined by their spectral absorption. The use of electrochemical detection (ECD) combined with HPLC is also very effective for analysis of electroactive or redox substances such as quinones, often allowing a simultaneous determination of reduced and oxidized forms. The simultaneous use of two working electrodes (dual-electrode cell) in ECD results in improved selectivity and sensitivity. In recent years, the use of RP-HPLC and ECD has frequently applied to the group of prenylquinones and their related reduction forms, in particular (70–77). Thus, anaerobic procedures have been reported for extraction and separation of plastoquinone A and its reduced form plastoquinol A (73, 74) using SEP Pak C_{18} cartridge (74). In this way the redox level of plastoquinone A in spinach chloroplasts could be determined with dual-electrode ECD. Further analyses include applications to prenylquinones of the ubiquinone (70, 71, 75, 76) phylloquinone or α-tocopherylquinone (70, 71, 75) type, in special cases using glassy-carbon working (73) or porous graphite working (75) electrodes and using the oxidative mode for determination of the reduced forms (ubiquinols, tocopherols, etc.). (For further applications see (13)).

Only a few reports are available on the application of Gas Liquid Chromatography (GLC) to natural quinones (78–84), involving mostly capillary columns and, for all samples, detection by flame ionisation (FID). Direct GLC analysis is restricted to the few thermally stable naturally occuring aglycones which are sufficiently volatile, e. g. p-benzo- and 1,4-naphthoquinones (81, 83, 84), whose gc behaviour was investigated using a traditional packed column with a OV-17 or SE-30 stationary phase (81). Derivatization of aglycones is usually needed to increase their volatility (78, 79, 80, 82). Different silylation procedures have been reported for anthraquinones, anthrones and tetrahydroanthracene derivatives. Thus, excellent separations of a mixture consisting of 37 naturally occuring anthraquinones within 25 min (80) and of 18 anthraquinones and related compounds from Cassia obtusifolia seeds within 15 min (82) have been achieved using a bonded phase fused silica capillary column and capillary GLC-MS. The use of computerised GC-systems has the advantage, that in cases where retention times are the same, compounds can be identified by their mass spectra or specific ions.

From special chromatographic methods, capillary column supercritical fluid chromatography (SFC) provides high resolution separations of commercial anthraquinone dyes, using supercritical n-pentane as mobile phase and UV detection. SFC using a packed microbore column combined with Fourier

transform infrared spectrometry with an automatic diffuse reflectance interface was recently developed and ensures high sensitivity. It was applied to acenaphthene quinones using supercritical carbon dioxide/MeOH as the mobile phase. A growing interest is being manifested in SFC using open-tubular or capillary columns in natural product isolation (85).

In biological systems quinones can also be detected by using electrochemical techniques, also without previous chromatographic separation, i. e. by cyclic voltammetry and polarography (1, 6). The latter method uses a dropping mercury electrode. Typical concentrations of the quinones are in the mM range. EC methods have been mainly applied to the analysis of natural quinones or the investigation of quinonoid compounds as models for biochemical reactions, such as the simultaneous determination of reduced and oxidized prenylquinones (see also: Voltammetric measurements of quinones (6)).

IV. Recent Developments in Analytics of Pharmacopoeial Emodin Drugs

This special section of Ch. III deals with recent developments in the analytics of official pharmacopoeial emodin drugs derived from Aloe, Rhamnus, Rheum and Senna spp. or of their single anthranoid constituents, the subject matter of which has also been dealt with in the symposia on anthraquinone laxatives 1976 (86), 1980 (17) and 1985 (14). Useful discussions and literature references may be taken from the new Ph. Eur./German Pharmacopoeia (DAB 9) commentary (15). Therefore, the topics which appear in those reviews, shall not be discussed. First the familiar Borntträger reaction, which has been used widely for a long time for the detection and estimation of anthraquinone derivatives, has been replaced by magnesium acetate in both the Ph. Eur. and DAB 9 (for a discussion see (15, 17)). In the meantime, a cupric acetate spray reagent for TLC of anthraquinones was recommended which should give a more stable colour (87). Recently, a new selective pharmacopeial TLC test for anthrones was proposed using nitrobluetetrazolium(NBT)-reagent (94). To differentiate between fresh and aged frangula or cascara bark the Ph. Eur. tests for the presence of unwanted reduced anthrones by means of TLC: Anthrones react with 4-nitrosodimethylanilin (NDA)/pyridine with the formation of grey-blue azomethines. Some commentaries (15) and reference books critisize the application of this reagent because of its toxic and carcinogenic properties and it is recommended that it should be handled with care. Now we propose the use of a new selective reagent in the place of NDA. By spraying with the fade yellow nitrobluetetrazolium reagent, an aubergine-violet biformazan is reductively formed in the presence of the undesired anthrones. The anthrones are oxidized to the bianthrones and small quantities of anthraquinones.

In TLC some new techniques for the separation, detection and determination of 1,8-dihydroxyanthracene derivatives have been used: thermofractography (for discussion see (17)); two-dimensional TLC using enzymatic or acid hydrolysis of Rhamnaceous anthraglycosides on the TLC plate (for discussion see (13)); identification of anthranoid drugs with two-dimensional TLC using the "TLC-reaction-box-process" and acid or oxidative ($K_2Cr_2O_7$) hydrolysis (88); a comparison of circular and linear overpressured layer chromatography by means of emodin-type aglycones and a systematic approach for optimisation of the mobile phase using the "Prisma model" (36, 89) (cp. Ch. II); determination of emodins from Rhamnus purshianus cell culture (90), of aloe-emodin glycosides (91) or sennosides (HPTLC-plates) (92) from Senna, of barbaloin-mixture from Aloe by TLC densitometry (93). The anthraquinone aglycones from plant cell cultures of Rhamnus frangula (132) and Rhamnus purshianus (133) were separated and quantitatively determined by an isocratic RP-HPLC procedure using a MeOH-H_2O-HCOOH solvent system. A further HPLC application shows the separation of an artificial mixture of typical plant emodins by ion-pair RP-HPLC (gradient elution with MeOH/H_2O/NBu_4-phosphate) (111).

Since the above mentioned symposia, a series of new compounds has been isolated from emodin drugs, which serve in part for the identification and purity tests, and in part for the quantitative assay of active components, and hence are important for the determination of activity and toxicology.

Aloe barbadensis, Aloe capensis (Aloes)

In Ph. Eur. TLC Barbados aloes obtained from A. barbadensis is identified by a violet band just below the band due to aloin, which appears after heating for 5 min at 110°C. The substances of this violet zone were identified as 7-hydroxyaloins A and B (48, 95) as well as 8-O-methyl-7-hydroxyaloins A and B (96) (the old "isobarbaloin"). Further violet bands appearing above the aloin bands, correspond to various cinnamoyl esters of these four 7-hydroxyaloins (96). Watersoluble oxidation products of these 7-hydroxyaloin-type glycosyls (48) are also responsible for the violet coulored solution in the bromine test tube-reaction ("Rosenthaler reaction") used as an identification test in Ph. Eur. and, in addition, in several other chemical tests (e.g. nitrous acid test; Klunge's isobarbaloin test). The pale yellow precipitate in this bromine water test consists of the diastereomeric 2,4,5,7-tetrabromaloins A and B (29) (more than 90%). In Cape aloe obtained from A. ferox the so-called "periodate positive substance" (98) was identified as 5-hydroxyaloin A (99). This extremely labile compound is the most specific, characteristic marker substance in TLC proof of Cape aloes and may easily be detected in an unique reaction as violet zone after spraying with $NaIO_4$-reagent. Until now, it is the only C-glucosylderivative, which has not been found as diastereomeric pair, but only in one configuration (99) (cp. Ch. V.). Furthermore, TLC analysis of the official and other aloes could considerably be optimised by the use of a mobile phase on the basis of $CHCl_3$-MeOH-H_2O or $CHCl_3$-MeOH-iPrOH-H_2O, which allows a separation and resolution of the diastereomeric aloins, 5-, 7- and 10-hydroxyaloins (see also Cascara) as well as their esterified derivatives (48, 100). Thus, at the moment 12 pairs of anthrone and oxanthrone C-glucosyls can be detected and separated in this way. For both, qualitative and quantitative determination of the various diastereomeric C-glucosyls HPLC (RP18; MeOH-H_2O) is the method of choice, as shown for Cape aloe (aloins A/B) (27, 28) and Curacao aloe (48) (aloins and their various 7-hydroxy-derivatives A/B).

Rhamni purshiani cortex (Cascara bark)

From Cascara bark a new anthranoid principle is presented here for the first time, namely the oxanthrone-C-glucosyl compounds of the 10-hydroxyaloin-type and their corresponding 8-O-glucosides of the 10-hydroxycascaroside-type, which must now also be considered in the qualitative (e.g. TLC) and quantitative (e.g. spectralphotometry) assay of Rhamnus

purshianus. In this context, it is the unravelling of the mystery of the old Schindlers' oxanthrone-O-glucoside (101) of frangula-emodin from Cascara, about which several authors have commented, e.g. in (102): "one should like to delete the oxanthrone and its O-glycoside from the list of active principle of Cascara", and it was assumed "that is really a normal anthrone-C-glycoside". We have been able to isolate and identify this compound as the diastereomeric 10-C-Glucosyl-frangulaemodin-oxanthrones (103). In addition, the new 10-hydroxycascaroside-type was detected, altogether 8 new compounds, neither of which has been previously found by analysts. These may have wide implications for analytics, pharmacology/physiology, stability of Aloe and Cascara formulations, chemotaxonomy and biosynthesis.

Frangulae cortex (Frangula bark)

The barks of Rhamnus fallax and Rhamnus catharticus are often encountered as adulterants of the official Frangula and Cascara bark in commercial products. In the chromatogramm of Frangulae cortex according to Ph. Eur. a red zone should not be present at Rf 0.1, which is indicative of the adulterant Rh. fallax. The corresponding substance could be identified as an unusual, chemotaxonomically interesting 3-O-apiosyl-8-C-methyl-anthraquinone-7-carboxylic acid, rhamnalpinoside (104). It was shown (69), that this TLC examination of Rh. fallax allows no positive, unambiguous identification, since rhamnalpinoside also occurs in Frangula bark. An improved TLC detection of the characteristic flavonol triglycosides xanthorhamnin as a yellow fluorescing spot using the diphenylboryloxyethylamine-reagent has been described as a proper method for the identification of the Rh. fallax bark (69). Furthermore the characteristic five main anthra- and naphthoquinones (2-methoxystypandrone), the six main anthraquinone glycosides and the above mentioned 8-C-methyl-anthraquinone-7-carboxylic acids rhamnalpinoside and its aglycone were analysed by HPLC using a MeOH/H_2O-gradient system and RP18 silica gel (69). Both adulterants, Rh. fallax and Rh. catharticus, contain three 2-hydroxyemodins, namely 2-hy-droxyfrangulaemodin (alaternin) (105), 2-hydroxychrysophanol and 2-hydroxyphyscion, which do not occur in the official barks (106). In TLC differentiation (106) these show a violet-purple colour in the Bornträger reaction due to the alizarin-type substitution, while the "normal" emodins give a red colour. In the TLC of Ph. Eur. the second adulterant Rh. catharticus is examined under UV light at 365 nm, where the chromatogramms should not show zones with an intense blue fluorescence, which are from Rh. catharticus bark. These compounds were identified as the naphthalide primeverosides sorinin and 6-methoxy-sorinin (107) at Rf 0.25 and as their corresponding monoglucosides of sorigenin and 6-methoxy-sorigenin (107, 108) at Rf 0.4.

Rhei radix (Rhubarb root)

Adulteration of official Rheum palmatum and Rheum officinale has been observed now and again occasionally, especially with R. rhaponticum, R. rhabarbarum and R. webbianum, all of which contain considerably less of the anthranoids than the official drugs. The TLC test given in DAB 9 for rhaponticin-containing Rheum sp. is reliable and no longer presents an analytical problem. Drugs from Pakistan and China appear to have different chemical races depending on their geographical origins: thus, in TLC according to Ph. Eur. no aloe-emodin and rhein are found (109). The amounts of single emodins of these Rheum drugs from different sources were quantitatively determined after acid hydrolysis. The results lead to a reduction in the minimum amount from 3.5 to 2.5% in the pharmacopoeial requirements. For further analytical details see commentary DAB 9 (15).

Sennae folium (Senna leaf) / Senna fructus (Senna fruits)

For TLC identification and differentiation of the two official senna drugs two different naphthalene glycosides, tinnevellin glycoside or 6-hydroxymusizin glycoside can be used as markers for Cassia angustifolia and C. senna (110). For quantitative analysis of sennosides TLC densitometry by fluorimetric detection and by in situ reduction with $NaBH_4$ was used, which attained a detection limit of 10 ng (92). A radioimmuno assay procedure has been described for the quantitative determination of nanogram-amounts of sennosides B and rheinanthrone-8-glucoside in extracts of Cassia angustifolia (112). For complete separation of all sennosides and their quantitative determination HPLC is the method of choice (113). Different systems have been proposed for sennosides from senna or rheum drugs, using reversed phase chromatography, especially with ion pair chromatography (picrate buffer (114), cetrimide (115) or tetra-n-heptylammoniumbromide solutions (116)) or dimethylamino-bonded silica gel columns (117, 118). For a recent review of the chemistry of the senna drug see (113).

This review of the advances in analytical methods shows, that the current quality control of emodin drugs and pharmaceutical preparations can exclude every adulteration, and a standardized composition of active components in commercial preparations can be guaranteed. At the same time the perfection of the analytical techniques demands a corresponding determination of activity and toxicology in the pharmacological sense. It is necessary, that rarely considered questions of synergy, the additive or potentiating activity of anthranoid drug mixtures and their naturally occuring adjuncts should be attacked by the pharmacologists, in order to correlate between the chemical composition of a drug and its activity. A further question of interest is, whether and how far the modern HPLC technique can or should replace the current conventional methods of pharmacopoeias.

V. Structure Determination

To establish the nature of quinonoid compounds and the number of substituents, the substitution pattern and their stereochemical features, spectroscopic methods, often in combination with chemical reactions, have proved extremely valuable. The advances made in recent years in quinone structure studies can be expressed in terms of the revision of structures of known compounds and the elucidation of structures of novel compounds, as well as in terms of additional information obtained by applying new methods and techniques. More recent structural investigations of quinonoids reflect extensive use of carbon-13 NMR techniques (1, 12), but also individual techniques like oxygen-17 NMR (1, 119) or gas phase UV photoelectron spectra (PES) (120) have been applied to quinones (for a comprehensive discussion of special structure types, see (1, 10, 12). In this section by means of the structure elucidation and stereochemical studies of C-glucosylanthrones of the aloin type and C-glucosyloxanthrones of the 10-hydroxyaloin-type, we show which methods are used today for the solution of such problems and which information can be obtained from them: X-ray cristallography, molecular

modeling systems, CD spectra, NMR-techniques, soft ionization MS techniques and combined LC/MS.

Aloin, which is known to be present in at least 68 Aloe species, may be regarded as parent structure for other naturally occuring diastereomeric 10-glucosylanthrones. Almost 140 years after the first isolation of aloin from the world-wide used drug Aloe barbadensis (121), the absolute configuration of aloin B at C-10 has now been established (8a) by an X-ray structure analysis as 10R, 1'S, and thus that of aloin A as 10S,1'S (see fig. 1). The instability of the diastereomers in solution had hitherto prevented the growing of single crystals. The A and C rings in the 1,8-dihydroxyanthrone system may be described as planar within the limits of experimental accuracy. Ring B, on the other hand, has a "sofa" or "envelope" conformation, in which C10 is raised out of the plane of the other five ring atoms by 0.29 (2) Å. The glucopyranosyl residue at C10 is in axial, the hydrogen atom in equatorial position. The torsion angles about the C10-C1' bond reveal deviations from the staggered arrangement of about 15°, which may be a result of repulsion between H5 and H2'. A conformational analysis by the use of a molecular modeling method based on force-field calculations indicates two stable conformations of the anthrone system, the extra- and intra-forms, the extra form of which is energetically preferred (8c) (see fig. 1).

Diastereomeric oxanthrone-C-glucosyls, in which C10 of the glucosylanthrones is oxidized, have now been detected as a novel anthranoid principle from Cascara bark, namely the 10-hydroxyaloin-type as well as their corresponding 8-O-glucosides of the 10-hydroxycascaroside-type (8c, 103). The configurations of these stereoisomers were established by an X-ray structure analysis of 10-hydroxyaloin A as 10R, 1'R, and it then followed, that the configuration of B is 10S,1'R (8b). The others were assigned from their CD spectra, as were the diastereomeric pairs of anthrone glucosyls (129): The opposite signs and differing intensities of the Cotton effects in the ranges 280–300 nm (strong Cotton effects) and 320–360 nm (weaker Cotton effects) in the CD spectra of both 10-glucosyl-anthrone and -oxanthrone derivatives indicate their opposite configurations at C-10. However, it is noteworthy, that unsymmetrical hydroxyl substitution of the 1,8-dihydroxyanthrone-chromophore leads to sign change of the Cotton effects in spite of identical absolute configurations (e.g. homonataloins (130); 7- and 5-hydroxyaloins). A conformational analysis by the above mentioned molecular modeling method (8c) shows that the conformations of aloins A and B as well as the 10-hydroxyaloins, are essentially identical. In addition, the 10-hydroxyaloins A and B simultaneously represent the main conversion products of the mild oxidation of the aloins A/B in vitro, for example in pharmaceutical aloe preparations (29). Thus, the recently published structure of 4-hydroxyaloin must be revised (30).

Unfortunately the (R,S)-nomenclature is not suitable for giving a comfortable overview since the different substituents at centres of identical absolute configuration lead due to the priority rules to different (R,S)-designations. To retain a degree of clarity and comparability for the assignments at C-10 in each of the diastereomeric pairs we have taken the parent structures aloin A and B and related the structures of the other diastereoisomers to them. Thus, 10-hydroxyaloin A belongs to the A-series and 10-hydroxyaloin B to the B-series all assignments being referred to the position of the glucose on the anthrone skeleton. In table 2 the absolute configurations of 30 naturally occuring anthrone- and oxanthrone-10-C-glucosyl compounds are presented here for the first time, which were

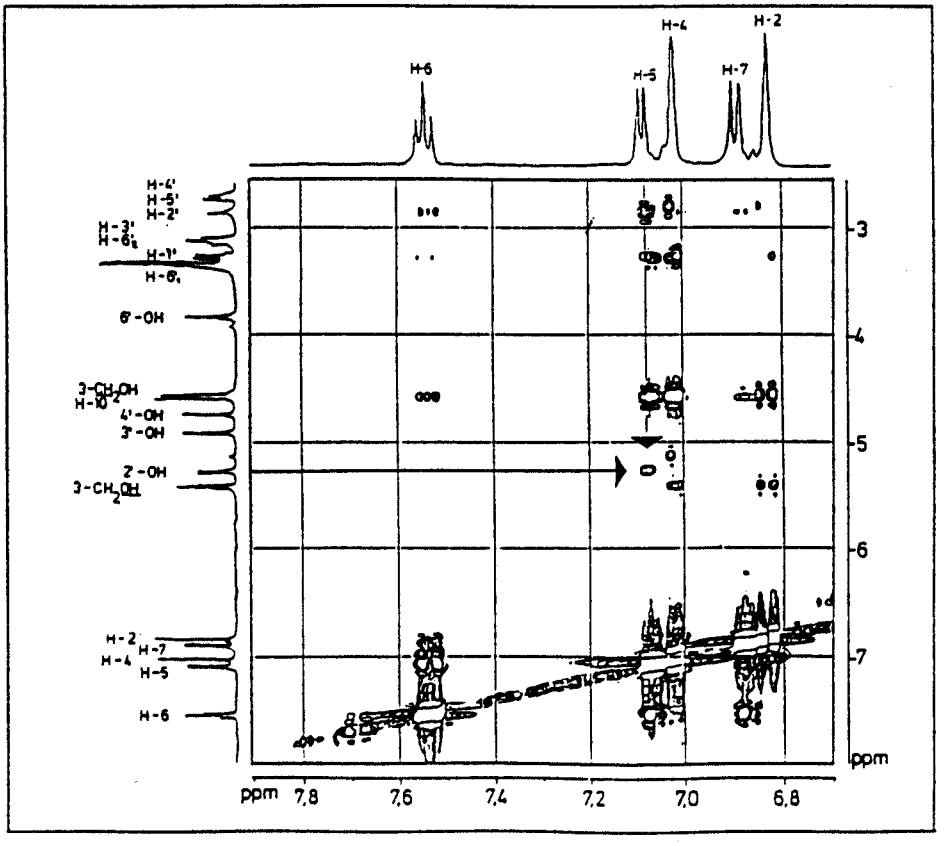
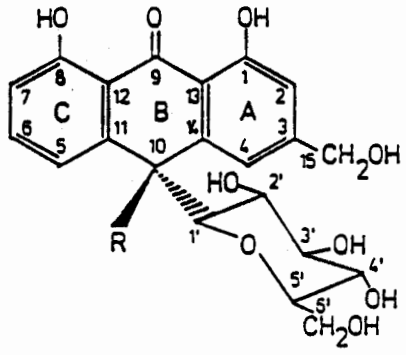


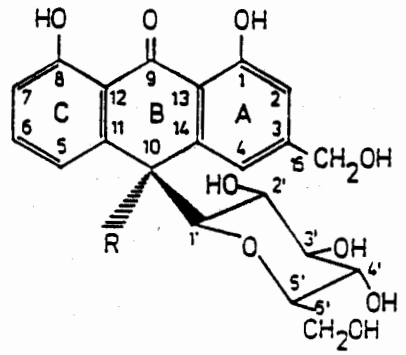
Fig. 2: Determination of the absolute configuration of Aloin B by two-dimensional ^{1}H NMR spectroscopy: The ROESY spectrum shows the repulsion between the glucosyl-2'-OH and the aromatic H-5 indicating a 10 R configuration

deduced by correlative CD or/and $^1H/^{13}C$ NMR spectroscopic data on the basis of the above-mentioned X-ray analyses.

The repulsion of H-2' resp. 2'-OH of the glucosyl residue by either H-5 (aloin B/10-hydroxyaloin B) or H-4 respectively (aloin A/10-hydroxyaloin A) of the anthrone system involves various implications on the structural analysis by NMR techniques: in the ^1H NMR spectra only the signals of the 2'-hydroxyl protons are readily distinguishable (124). The A-series always has the lower δ-values for the 2'-OH (e.g. 10-hydroxyaloin A: 5,5–5,6 ppm), the values for the B-series being shifted more strongly to the low field region (10-hydroxyaloin B: δ 5,8–5,9 ppm). In the case of 10-hydroxyaloin A and B the aromatic H-4 and H-5 signals have reversed δ-values, thus being useful parameters for a configurational assignment. NOE difference ^1H NMR spectra clearly show an interaction of 2'-OH and H-5 in the B-row, corresponding to the X-ray analytic result, and the opposite case an interaction to the hydroxymethyl at C3 resp. H-4 (A-row). Additionally, the signals of anomeric protons resonate in the same range as the other sugar ring protons and could only be identified by the application of the double resonance technique, as recently reported (124). This unambiquous assignment allows a simple determination of the β-configuration of the glucosyl residue in

Table 2: The absolute configurations of naturally occurring anthrone- and oxanthrone-10-C-β-D-glucosyl compounds, determined by CD [129] and $^1H/^{13}C$ NMR spectroscopic data [124] correlated on the basis of X-ray analyses [8a,b]





C-Glucosylanthrones (R=H)

Aloin A-Type	Configuration		Aloin B-Type	Configuration	
	C-10	C-1'		C-10	C-1'
(1) Aloin A*	S	S	(2) Aloin B*	R	S
(3) Aloinoside A	S	S	(4) Aloinoside B	R	S
(5) 15-Desoxyaloin A (Chrysaloin)	S	S	(6) 15-Desoxyaloin B	R	S
(7) Tetrabromaloin A	S	S	(8) Tetrabromaloin B	R	S
(9) 5-Hydroxyaloin A	R	S	(10) 5-Hydroxyaloin B	S	S
(11) 7-Hydroxyaloin A*	S	S	(12) 7-Hydroxyaloin B*	R	S
(13) 8-O-Methyl-7-hydroxy-aloin A*	S	S	(14) 8-O-Methyl-7-hydroxyaloin B*	R	S
(15) Homonataloin A	S	S	(16) Homonataloin B	R	S
(17) Cascaroside A	S	S	(18) Cascaroside B	R	S
(19) Cascaroside C	S	S	(20) Cascaroside D	R	S
(21) Cascaroside E	R	S	(22) Cascaroside F	S	S

C-Glucosyloxanthrones (R=OH)

10-Hydroxyaloin A-type	Configuration		10-Hydroxyaloin B-type	Configuration	
	C-10	C-1'		C-10	C-1'
(23) 10-Hydroxyaloin A	R	R	(24) 10-Hydroxyaloin B	S	R
(25) 10-C-Glucosyl-10-hydroxy-frangulaemodinanthron A	S	R	(26) 10-C-Glucosyl-10-hydroxy-frangulaemodinanthron B	R	R
(27) 10-Hydroxycascaroside A	R	R	(28) 10-Hydroxycascaroside B	S	R
(29) 10-Hydroxycascaroside E	S	R	(30) 10-Hydroxycascaroside F	R	R

* In addition, ten novel acetyl and cinnamoyl sugar esters (cp. table 1).

both aloins. Two-dimensional HH COSY (homonuclear) experiments were useful for determining the positions of various ester groups, e.g. in mono- and diacetylated or cinnamoylated 7-hydroxyaloins (126). The corresponding heteronuclear variant ^{1}H, ^{13}C correlated (HC COSY) shows direct attachments between hydrogen and carbon atoms and was used in combination with the one-dimensional ^{13}C DEPT spectra for unambiquous assignment of C signals of various aloins and hydroxyaloins, particularly of C-10 in anthrones and oxanthrones (125). An efficient two-dimensional NMR technique for differentiation of the stereoisomers is the recent introduced ROESY method (= rotation frame Overhauser experiment), which gives information on intramolecular distances analogous to the NOESY experiment (125). In contrast to NOESY, in the ROESY experiment in the spin-locked state the NOE effect is always positive and the technique can also be used at high field strengths. Thus, cross peaks indicative of the NOE effects and cross peaks indicative of ^{1}H-^{1}H exchange are useful parameters for assignment to the A- or B-row (Fig. 2). The complete ^{13}C NMR signals, till now not yet reported, are easily assignable by the inverse-correlation spectra in the recently introduced BIRD technique, showing a greatly increased sensivity compared to COLOC. Thus, the significantly different absorptions of C-4/5 in both types of stereoisomers A and B are further valuable parameters for the assignment of configurations (125).

Finally, the structure elucidation of 25 new, labile anthrone and oxanthrone derivatives occuring in free, esterified or glycosylated forms with the basic hydroxylsubstitutions in positions 5, 7 or 10 (cp. table 2), would not have succeeded without the application of soft ionization mass spectrometric techniques such as FAB, DCI or CI (isobutane or ammonia as reactant gas). Definite information can be obtained on the structure of the aglycone, of the nature or sequence of the sugars or the esterifying components. Furthermore, the use of high resolution and tandem mass spectrometry (MS/MS; Finnigan MAT HSQ 30 and MAT 90 QQ) based upon the dependence of the NCI mass spectra on the CI gas pressure (e.g. argon), allows a fast and reliable differentiation between the various isomeric 5-, 7- and 10-hydroxyaloins (127). The coupling of LC and MS (LC-MS) is gradually becoming an important analytical tool. For example, the complex mixture of a raw extract from Aloe barbadensis was analyzed with a BEQQ Hybrid mass spectrometer using LC/MS/MS technique with a moving belt interface (128). The results show that the characteristic substances aloin and 7-hydroxyaloin are very easily to identify and quantify by the parent scan of m/z 255 or m/z 271 after a neutral loss of 163 due to glucosyl elimination. Chromatographic optimizations are not necessary, since the high selectivity makes purification steps redundant. The use of MS/MS can considerably reduce analysis time by replacing the chromatographic separation by the selectivity of the MS/MS technique.

VI. Conclusions

Finally, if one attempts to evaluate the analytical methods which are at present available to us for quinones and other related natural compounds, then it is clear, that in spite of all the fascinating discoveries and developments there is no one best and universal method and there will probably not be one in the future. More importantly, it is the correct choice and combination of individual methods which lead to the best results. In spite of increasing automation human experience and intelligence will still always be the decisive factor in the analysis of natural products. One can expect that, because of the continual advances in chromatographic separation techniques and spectroscopical methods and the various combinations of these, other, sometimes surprising quinonoid compounds will soon be found in nature, which will be of importance in medicinal, pharmaceutical, biological, ecological or biochemical questions.

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Further 40 references on HPLC/TLC analyses of prenylbenzo- and -naphthoquinones including K-vitamins and 13 references of table 1 (DCCC isolation) may be obtained by the author.

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