I. Introduction

The purpose of this report is to review current knowledge and recent advances with respect to the isolation and analysis of naturally occurring 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 analyses (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 absolve 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 11 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 quinoid structural types or groups. The more than 1300 naturally occurring 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 (II, II'), e.g. the division "benzoquinones" contains 11 different subcategories. The main subject will be the analytical applications of the acetate-derived class of anthraquinones and the o-quinone chromophores (\(\alpha\)- and \(\beta\)-anthrones and \(\alpha\)- and \(\beta\)-anthronoquinones) as well as a few selected other quinoid and quinoid-derivative color forms like \(\alpha\)-hydroxy-, \(\beta\)-hydroxy-, \(\alpha\)-ceto-, \(\beta\)-ceto- and \(\alpha\)-amino-\(\beta\)-keto-quinones, and some closely related by-products.

For: Prof. Dr. Georg Schneider, Frankfurt, on the occasion of his 60th birthday.

Manuscripts of a physics lecture given on 27th October 1989 at the "International Symposium on Chromatography and Natural Products Analysis" in Aachen/Switzerland.
Fig. 1: Structural Formulas of the Aloe and 10-Hydroxyaloeine types.
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 procedure are based on this layer chromatograpphy (TLC) using silica gel and polyamide 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. However, obviously no particular solvent system can be recommended for all quinones.

For example, quinone isolation from marine organisms (24) 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), Lichrosorb Si-60, Polyamide (CC) and preparative liquid chromatography (low, medium, high pressure and flash chromatography).

Methods using a column-chromatographic principle are: preparative column-chromatography (LCCC), rotation isolar counter-current-chromatography (RCCC) and centrifugal counter-current chromatography (PCPC). Their applications and development are discussed below. Without considering chromatographic parameters, advantages, limits and disadvantages (for these considerations see sect. 5) it finally should be mentioned that the techniques presented here are not mutually exclusive but complement 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 matrices, carried out with the commercially available "Chromatotron" (anhraquinone (22), anthraquinone (23) and "Phortech" (anhydroquinones (24, 35), using sequential CTLC and centrifugal plates CC, indicate that centrifugal TLC has been employed only for the isolation of aglycones, which are in general easier to separate than quinone glucosides. In this context, to help in optimizing the mobile phase, the so called PRISMA- model, based on Snyder's solvent selectivity triangular, was employed (26). This model has already been used for classical and on-column pressure as well as HPLC.

The PRISMA technique is especially useful for ternary and quaternary solvent systems but less for binary systems. The most commonly used CTLC eluents are: Although often frequently separated on a preparative scale by means of CC using silica adsorbents. Columns packed with Sephadex LH-20 are also widely used, for example for the fractionation of artemisinic derivatives from Aslo (37) and Knospia spp. (38) or recently in the separation of closely related intermedoid quinones of the phenanthroic-type or plect- anthroic-type from Coleus and Salvia species (39).

A further example is the efficient separation of loible gua- cronfractinolos A and B, which was achieved in combination with MPLC (22). CC on XAD-2, 3-polyamine-dextran copolymer, has been used for the purification of anthraquinones from Rheum (40) and Rhus spp. (41). Some applications using superficial fluid chromatography (SFC) techniques for the isolation of commercial dyes, e.g. synthetic anthraquinones are discussed in (12). According to ref. (4) the term "pressure liquid chromatography" used here to include any method involving the application of pressure to a chromatographic column, as distinct from gravity-fed separations. Thus, all categories ranging from flash LC with 2 bar to preparative HPLC with 100 bar, and sample sizes from 100 mg to 1 kg can be included under this heading.

The simple techniques of flash chromatography, basically or air pressure driven, are used for a variety of pressure and short column
chromatography, allows particularly rapid and efficient separations, as is demonstrated by the isolation of hydroxylation intermediates from C. elegans (51) or naphthoquinones from Diopysus wuhsiensis (33) or antirheumatic extracts from Passoverpim (sp. 99).

Only a few papers are described using low pressure LC systems; a series of digitoxigenin glycosides from Coleus forskohlii has been isolated by chromatography on Lobar Si 60 columns with various hexane-ace tone mixtures (43). The MPE technique was also used on Lobar Si 60 columns with large internal diameters and require higher pressures, e.g., PLC 1 (5-200) to enable sufficiently high flow rates. PLC C 1 is a highly specific silica gel and is patented system containing MeOH:H2O:HCOH, in combination with Sephadex LH-20 open cell CC was employed for the preparative separation of high fructose fructosans from A and B, a closely related primary biophytosides of fresh Rubusus frangula Jackie (22). The complete separation takes no longer than 3 h, of which only 35 min. is required for the MPLC.

For as natural product isolation, preparative HPLC is still rarely used within quinones; one application of preparative HPLC by the radial compression system of WATERFROAT P 500, using RP 50 on octadecyl-bonded columns, is described for ethaquinone intomutin and quinones from fermentation broth of Actinomycopsis sp. (44) (sample sizes: 3-25 g; mobile phase: CH2Cl2:MeOH:H2O:CH3OH, 9:1:4:6). The presence of amino acids in the eluting solvents led to problems such as desorption of the columns and a shortening of column life. Furthermore, terpenylketo quinones and -quinones were obtained from the brown alga Cystoseira helianthae (45) by the radial compression HPLC (LChromprep Si 60; C18:EtOH 9:1:4:6).

All the techniques mentioned so far, to solid stationary phase or a liquid stationary phase fixed on an "intert" support, are used for the separation of individual components within a mixture. The separation is not limited to small molecules but supports various support-free counter-current chromatographic techniques based on liquid-liquid partition have been developed in the last decade (4). Among these DCC has established itself as a standard laboratory procedure for the separation of natural products. DCC also has been successful in the separation of various alkaloids and naphthyl derivatives. 21 applications are summarized in table 2. In our laboratory, as in the method of choice, DCC is used not only for the separation of various glycosides and -carcinins from Alloc and Cascarina, but also for recovering each 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 a- and B 7 (1%), which should formerly only be accomplished by HPLC (27, 58) or by fractional crystallizations (57). The solvent composition was governed primarily by cocrystallization of the partition coeffi- cients of both stereoisomers, using HPLC to determine the partition ratio of A and B in the two solvents recorded by the two photometers (A and B), the DCC method is sufficiently rapid to avoid problems of contamination. Compared to HPLC, the resolution of racemic or enantiomeric compounds is enhanced. HPLC techniques have found only very restricted application in the area of quinones. Variations chromatographic, dissimilar antitumor activity from the crude chromato- graphic extract of Spermacoce species cause brushes that have been successfully separated by HPLC using solvents based on CHCl3:EtOAc:MeOH:H2O (46). Lipophilic fraction of the crude chromatographic extract of the "inert" system n-hexane/ETOH/PY/CHCl3 in the second column (47). The method of fractionation is very critical comparison of Rf values DCC was shown for the separation of eluates (48); the solvent systems were only one fifth of the amount of elution (60). resolution was lower, seleno-should be separated. Centrifugal liquid-liquid pastic chromatography (CFC) with a Spheri- CPC apparatus has been used to perform rapid separations of antinutrients pigments from Passoverpim vermilion and nephoblast and from Cellolysin staminata by 49). The technique of horizontal flow-through coil plant centrifuge (CHFPC) was applied for the separation of some antinutrients in one step with an increase in the yield and their metabolites (50, 51) and for the isolation of the diastereomeric dihydroquinoine oxides and its hydrolys products (52).

III. Qualitative and quantitative Analysis

In this section the various chromatographic and, in short form, electrochemical methods used for detection and determi- nation of quinones are discussed. Recent developments in analysis of affinial pharmaceutical antinutrient drops or of their antinutrient constituents are considered in Ch. IV. Individ- ual applications in the quantitative determination of certain quinone compounds or type using specific techniques (mainly spectrophotometry and polarography) or chemical methods (mainly colorimetric determinations) may be taken from review articles (e.g. 1, 19, 11, 12, 53). With other organic molecules, quinones may be 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 tech- niques 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 insensible, simple, fast and effective method for the general characterization of quinones; it should be indicated, however, that some quinones are highly unstable in the dried state, especially when exposed to light, hence may be "ruling" due to the formation of hydroquinone, which, in turn, may be partly air-oxidized, when subjected to TLC (op. cit.). TLC is indis- pensable for the checking of a crude plant extract for quinones, particularly within the scope of the post-harvest analyses (Ch. IV), and often provides some structural information (1, 10, 11, 12). TLC is also very useful in determining fractions obtained in the preparative separation by other methods and in testing homogeneity. Fast chromatography, TLC is of great help in the
Table: Quinoides and their Reactions Forms isolated by DCCC

<table>
<thead>
<tr>
<th>Quinoides</th>
<th>Solvent system</th>
<th>Plant sources</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubescence anthracinae</td>
<td>CHCl₂-MeOH-H₂O</td>
<td>Gallium molina</td>
<td>Inoue et al. (1979)</td>
</tr>
<tr>
<td>Lucifer primrose</td>
<td>CHCl₂-MeOH-H₂O</td>
<td>Morelia citrinella</td>
<td>Inoue et al. (1981)</td>
</tr>
<tr>
<td>Aglycone 1 and glycosides</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Kubia cordifolia</td>
<td>Hikawa et al. (1983)</td>
</tr>
<tr>
<td>Ruberubic acid</td>
<td>n-Hexane-CH₂OH-H₂O-CH₂OH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhamnose anthracinae/sephalides</td>
<td>n-BuOH-MeOH-H₂O</td>
<td>Rhamnus catharticus</td>
<td>Rauwald, Jun (1983)</td>
</tr>
<tr>
<td>Serrut, 6-methoxysoyan and their monoglycosides</td>
<td>CHCl₂-MeOH-H₂O</td>
<td>Rhamnus catharticus</td>
<td>Rauwald (1987)</td>
</tr>
<tr>
<td>Emoeprinone-erwina/genticisic/nico/ -glucose</td>
<td>CH₂Cl₂-MeOH-H₂O-HCOOH</td>
<td>Rhamnus falax</td>
<td>Vieichos, Rauwald (1987)</td>
</tr>
<tr>
<td>Alaslerin, 2-methoxystapanone (aglycone)</td>
<td>n-Hexane-MeOH</td>
<td>Rhamnus falax</td>
<td>Mieting, Rauwald (1984)</td>
</tr>
<tr>
<td>Rhamnaisoide and its aglycone</td>
<td>CHCl₂-MeOH-H₂O-HCOOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiantrema glycosides</td>
<td>CHCl₂-MeOH-CH₂OH-H₂O</td>
<td>Rheum sp.</td>
<td>Oghara et al. (1976)</td>
</tr>
<tr>
<td>Sennoside A/B</td>
<td>CHCl₂-MeOH-H₂O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>anthracene 4-10-estrenones</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1988)</td>
</tr>
<tr>
<td>Alkene A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1983)</td>
</tr>
<tr>
<td>8-5-O-Coumaryls-alken A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe feroxita</td>
<td>Rauwald et al. (1986) (126)</td>
</tr>
<tr>
<td>7-Hydroxyalous A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald, Diemer (1987) (126)</td>
</tr>
<tr>
<td>8-5-O-Coumaryls-7-hydroxyls-</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald, Diemer (1987) (126)</td>
</tr>
<tr>
<td>A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1983)</td>
</tr>
<tr>
<td>8-5-O-Chloryl-5-hydroxyals A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1983)</td>
</tr>
<tr>
<td>6-5-O-Coumaryls-7-hydroxyals A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1983)</td>
</tr>
<tr>
<td>Homoteral A</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1983)</td>
</tr>
<tr>
<td>8-Hydroxyals A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1983)</td>
</tr>
<tr>
<td>5-4-hydroxyals A/B</td>
<td>CH₂Cl₂-MeOH-H₂O</td>
<td>Aloe barbadensis</td>
<td>Rauwald (1983)</td>
</tr>
</tbody>
</table>

Analytical HPLC is now a well-established, powerful and predominant technique useful for the separation of numerous types of plant quinones. HPLC has rapidly been employed for quantitative determination, purity verification of isolated compounds or for chemocognominal comparison of quinoid compounds and will in the near future certainly be pharmacologically interesting.
be become also more popular as a method for their isolation on a semi-preparative or preparative scale (cp. Ch. 11). Regarding the nature of column packing materials, in most of the literature reviewed here, separations on silica gel columns, mostly reversed phase columns (C8 or C18) have been used and only few on amino-chemically bonded silica gel or on the more polar phosphonate bonded phase HPLC. The use 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 allows an easy characterization of quinones. Another interesting application shows the simultaneous detection and determination of reduced glucouronidation products (12μm, 320nm) and oxidized glucouronidation products (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 quinoid groups, excluding the 1,8-dihydroryanthraquinones (see (Ch. IV), are presented in a keyword form to demonstrate the comprehensive use and possibilities of this technique for all groups of quinoloids: Rubiaeic acid arantquinones (15), particularly from Rubia spp. (58), insect pigment quinones of the kermesid-acid type (e.g. from Coccus cacti (59)), using the eluent MeOH/H2O/HCOOH and RP-18 material; reduced tetrahydroanthraquinones from Alternaria torm (60); hydroxyrhamnogen (61, 62); pigments from fungi (3) (terpenylquinones, fettuquinones etc.); plasmaticy antibiotics (56); anthracycl- one derivatives of the desoxoquin or daunorubon-type (57) or the new ellorcin type from Streptomyces divaceus (63); diol eluents; gradient elution with anisotine and 0.1% phosphoric acid. The anisotine type dienoid quinones from Plectranthus sanguineus have been assayed by non- aqueous cation-exchange HPLC using a Polygal 10-SCX column, and gradient elution (59). For the analysis of phytosterapeutically used naphthobenzothianone derivatives of the hypericin type from Hypericum perforatum herb an ion pair chromatographic method with gradient elution (eluted with (MeOH/ EtOAc-Na2HPO4 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 lawson-type naphthoquinones from a lignin extract of Diospyros texanae have been separated using 1% acetic acid in n-hexane as solvent and a CN bondedphase (66). A zebrole stationary phase and MeOH/H2O as eluent were used to examine the chromatographic behavior of several naturally occurring naphthoquinones from Bignoniaceae, e.g. Rauv- alica 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 (covelent trelease, hexane-isopropanol: isoelectric and gradient systems) which resolves α-β-lapachone and allom- gues well, whereas the quinols lapachol and isolapachol are reduced. A naphthoquinone, 2-methoxyxyanonone, from Rhamnus falax were assayed together with the characteristic 1,8-dihydroryanthraquinones (69) (RP18; gradient elution with MeOH/H2O and MeOH). Most analytical work on naphthoquinones has been confined to the highly lipid-soluble quinones, the K-vitamin and the prenylbenzoquinone derivatives (2, 5, 9) (ubiquinones, plastoquinones, tocotquinones), 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 review 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, in the groups of K-vitamins and L-β-tocotquinones, a new simple and selective spectrophotometric determination of menadione and menadione 4′-sulfate has been developed for the assay of photostable vitamin K preparations in Japanese Pharmacopeia using 3- ethylthiodihane (13).

In the HPLC procedures so far described, quinones are determined by their spectrophotometric detection after electrochemical detection (ECD) combined with HPLC is also very effective for analysis of electroactive natural 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 prenoliquinones and their related reduction forms, in particular (70-77). Thus, anodic and cathodic procedures have been reported for the extraction and separation of plastoquinone A and its reduced form plastoquinol A (73, 74) using SEP Pak C18 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 prenoliquinones of the ubiquinone (70, 71, 75, 76) plastoquinone or α-tocopherol-quinone (70, 71, 73, 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 ionization (FID). Direct GLC analysis restricted to the few thermally stable naturally occurring agly- como which are sufficiently volatile, e.g. p-benzene and L-4-naphthoquinone (81, 83, 84), whose behaviour was investigated using a traditional packed column with a OV-17 or SE-30 stationary phase (81), spectrophotometric determination of aglycone was usually needed to increase their volatility (78, 79, 80, 82). Different stylation procedures have been reported for arbutquinones, anthrones and tetracyclazole derivatives. Thus, econom- ical separations of a mixture consisting of 37 naturally occurring arbutquinones within 25 min (80) and 18 arbutquinones and related compounds from Costa oxidifolia seeds within 13 min (92) have been achieved using a bonded phase (fused silica capillary column and capillary GLC-MS. The use of computer- assisted 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 new infor- mation on the separation of commercial arbutquinone dyes, using supercritical n-entane as mobile phase and UV detection. SFC using unpacked columns is more suitable for the isolation of naphthoquinones.
IV. Recent Developments in Analysis of Pharmacopoeial Enodin Drugs

This special section of Ch. III Arznei with recent developments in the analysis of official pharmacopoeial enodin drugs derived from Aloe, Rheum, Rhus and Senna spp., or of their single anthraquinone 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) comment (13). The referees, the topics which appear in those reviews, shall not be discussed. First the familiar Biermann 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 cinnamyl 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 anhydrophenolic was proposed using microburet glass cuvette (A) (64) with the formation of blue-grey azoemine. Some commentaries (15) and reference books criticise the application of this reagent because of its toxic and carcinogenic properties and it is recommended that it should be handled with care. We now propose the use of a new selective reagent in the place of NOA/NOB reagent: by spraying the white yellow microturbidity reagent instead, the blue violet-brown emulsion is reversibly formed in the presence of the undetermined anthraquinones. The emulsions are oxidized to the bisthiochromes and small quantities of anthraquinones.

In TLC, some new techniques for the separation, detection and determination of 1,8-dihydroxyanthraquinone derivatives have been used: thermography [for discussion see (17)], two-dimensional TLC using enzymatic and acid hydrolysis of Rheum anthraquinones on the TLC plate (for discussion see (13)); identification of anthraquinone drugs with two-dimensional TLC using the "TLC-reaction between process" and iod or quinalizarine (15); hydrolysis (84); a comparison of similar and a form of overpressure layer chromatography by means of emodine-type aglycones and a synthetic approach for quantification of related anthraquinones in "Phytotherapie" (28, 30) (cf. Ch. Il); determination of emodin from Rheum purpureum on culture (90), of aloeside in aloes (91) and sesamin (HPTLC-1000) from Sena, of barbital in mixture from Aloe by TLC-densitometry (32). The anthraquinone aglycones from plant cell cultures of Rheum flavum (21) and Rheum purpureum (13) were separated and quantitatively determined by a new HPTLC procedure using a silica gel 60-H-OCOCHO solvent system. A further HPLC application shows the separation of an artificial mixture of typical plant pigments. These determinations are carried out in gradient elution with MeOH-OH-DEA-phosphate (11). In the above-mentioned symposia, a series of new compounds has been isolated from enodin drugs, which serves for the identification and purity tests, and in part for the quantitative assay of active components, and hence is important for the determination of activity and toxicology.

Aloe barbadensis, Aloe capensis (Aloes)

In Ph. Eur. TLC barbodin aloes obtained post A. barbadensis is identified by a violet band just below the band due to aloes, which appears after heating for 5 min at 110°C. The substances of this violet zone were identified as 7- and 8-methoxy aloes and B (48, 95) as well as 8-methyl-7-hydroxy aloes A and B (96) (the old "isosadabarin"). Further violet bands appearing above the violet bands, correspond to various cinnamyl esters of these four 7-hydroxy aloes (96). Water-soluble oxidation products of these 7-hydroxy-typc glucosides (48) are also responsible for the violet-touched solution in the bromine test reaction ("Holstein reaction") used as an identification test in Ph. Eur. and, in addition, in various other chemical tests (e.g. nitrous acid test; King's isobarbital test). The pale yellow precipitate in this bromine test water extract consists of the diastereoisomers 2,5,7-tetrahydroxy A and B (29) (now 90). In Cips Aloes obtained from A. febrifugum the so-called "isosadabarin" (98) was identified as 5,8-dihydroxy aloes A (99). Some extremely rare compound is the most specific characteristic marker substance in TLC-plot of Cape aloes and may easily be detected in an unique reaction as violet zone after spraying with NaNO2 reagent. Until now, it is only the C-glucoside derivative, which has not been found as dihydroereine, but in one configuration (99) (cf. Ch. V.). Therefore, further analysis of the official and other aloes could be considerably improved by the use of a modified phase on the basis of C18-reversed phase on silica or Chirabil-Hypersil pOrO-H2O, which allows a separation and resolution of the diastereomeric aloes, 5, 7- and 9-hydroxy aloes (see also Cascaró) as well as their esterified derivatives (84, 100). Thus, at the moment 26 pairs of anthrone and oxaanthone C-glycoside can be detected and separated in this way. Both, qualitative and quantitative determination of the various diastereomeric C-glycosyl HPLC (RP18; MeOH-H2O) is the method of choice, as shown for Cape aloes (aloes A/B) (27, 28) and Cascaró aloes (48) (aloes and their various 7-hydroxy derivatives A/B).

Rheum purpureum cortex (Cassia bark)

From Cassia bark a new anthraquinoid principle is presented here for the first time, namely the oxaanthone C-glycosyl resin (Cassia cortex type) and their corresponding 2-n-oligosidoS of the 2,8-dihydroxy-taucaroside-type, which may now also be considered in the qualitative (i.e. TLC) and quantitative (e.g. u.v-spectrophotometry) assay of Rheum.
In this context, it is the unravelling of the mystery of the old Schindler's oxantrone-O-glucoside (101) of fran-
gulace-emodin from Cassara, about which several authors have commented, e.g. in (103): "one should not judge the oxantrone and its O-glucoside from the active principle of Cassara", and it was assumed "that is really a normal antronone-C-glucoside". We have been able to identify this compound as the diastereomeric 10-C-Glucosyl-
frangulanolide-oxantrone (103). In addition, the new is-
hydronorflavonoid-tye structure was detected, altogether 11 new com-
ounds, which of them has been previously found by anal-
ysis.

These may have wide implications for analyses, phar-
cology, physiology, pathology of Aloe and Cassara formula-
tions, chemoraxonomy and biochemistry.

Frangulae cortex (Frangula bark)
The bark of Rhusbaum fassus and Rhamnus catharticus are often encountered as adulterants of the official Frangula and Cassara bark in commercial products. In the syonogram of Frangula cortex according to Ph. Eur. the red zone should not be present at Rf 0.1, which is indicative of the adulterant Rhamnus fassus. The corresponding substance could be identified as an unusual, chemoraxonomic interesting 3-O-apiosyli-
4,6-methyl-androstenedione-2,7-carboxylic acid, rhamnupinone-
(104). It was shown (69), that this TLC examination of Rh.
frangulae fails to positive, unambigious identification, since
franguladin A also occurs in Frangula bark. An improved
TLC detection of the flavonol flavonolides xanthohumolin in a yellow hşanessing spot using the splenify-
vonolxylose-vinylamine-reagent has been described as a proper
method for the identification of the Rh. fassus bark (59).

Furthermore the characteristics five main antrac- and naphtho-
quinones (2,7-dihydroxy-3H-xanthone, 2,6-dihydroxy-7H-
xanthone, 4,6-dihydroxy-2H-xanthone, 7,8-dihydroxy-2H-
xanthone, and 2,6-dihydroxy-3H-xanthone) have been described as a
proper method for the identification of the Rh. fassus bark (59).

It shows a violet-purple colour in the Borntraeger reaction due to
the xantieric-type substituio, while the "normal" emodins
give a red colour. In the TLC-comparisons, the second adulerant
Rhamnus catharticus is examined using UV light at 365nm, where
the chromatograms should not show zones with an intense
blue fluorescence, which are from Rhamnus catharticus. These
components were identified as the naphthylindene-2-carboxyl-
trione and 6-methyl-tartazine (107) at Rf 0.25 and as their
respective monoacetates of sorbins and 6-methoxy-
sorbins (107, 108) at Rf 0.4.

Rheia radix (Rheabark root)
Adulteration of official Rhei palmatum and Rhei officinalis has been observed now and again occasionally, especially with R. rhaponticum, R. rhabarbarum and R.
scorboracum, all of which contain considerably less of the
anthraquinons than the official drugs. The TLC test given in DAB
9 in the pharmacopoeia of Rhenland is reliable and no longer presents an analytical problem. Drugs from Pakistan
and China appear to have different chemical races depending on
their geographical origin: when, in TLC comparing to Ph.
Eur. no antronone and their are found (69). The amounts

of single emodic of these Rhum drugs from different sources
were quantitatively determined after acid hydrolysis. The
results lead to a reduction in the minimum amount from 0.5 to
2.5% in the pharmaceupical requirements. For further analyti-
cal details see commentary DAB 9 (15).

Sennae folium (Senna leaf) / Senna fruticis (Senna fruits)
For TLC identification and differentiation of the two official
senna drugs two-different naphthoquinones, inervinelline
ɡlucoside and 6-hydromethylglucoside can be used as mar-
kers for Cassia angustifolia and Senna (110). For quantita-
tive analysis of sennosides TCL densimetry by fluoroisometric
detection and by in situ reduction with NaBH₄ has been used, which
attains a detection limit of 10ng·92. A radiogram using
module procedure has been described for the quantitative determi-
ation of sennoside-amounts of sennoside B and their
anthraquinone-8-glucoside in extracts of Cassia angustifolia (112).

For complete separation of all sennosides and their quantita-
tive 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 (pizear buffer (114),
eceizidim (115) or tris-a-herp)Hembrum bicicelate solutions
(161) or dimethyamined-bonded kileg silica gel (117, 118).

For a recent review of the chemistry of the senna drugs see (113).

This review of the advances in analytical methods shows that,
the current quality control of emodin drugs and pharmaceu-
tical preparations can exclude all adulteration, and a standard-
ed composition of active components in commercial prepa-
rations can be guaranteed. At the same time the perfection of
the analytical techniques demands a corresponding determina-
tion of activity and toxicology in the pharmaceupical sense. It
is necessary, that fairly considered questions of synergy, the
discretion or potentiating activity of anthranoid drug mixtures
and their naturally occurring adjuncts should be adressed by the
pharmacologists, in order to correlate between the chemical
composition of a drug and its efficacy. A further question of
interest is, whether and how far the modern HPLC technique
can or should replace the current conventional methods of
pharmacopoeia.

V. Structure Determination
To establish the nature of quinoid compounds and the
chemical pattern and their stereomeric features, spectroscopic
methods, often in com-

bination with chemical reactions, have proved extremely
valuables. The advances made in recent years in quinoid
structure studies can be expressed in terms of the tension of
structures of known compounds and the elucidation of struc-
tures of novel compounds, as well as in terms of additional,
information obtained by applying new methods and tech-
niques. More recent structural investigations of quinoids
reflect extensive use of carbon-13-NMR techniques (1, 12), but
also individual techniques like oxid-17 NMR (1, 119) or gas
phase UV absorption spectra (PED) (120) have been applied
to quinoids (for a comprehensive discussion of special
structure types, see (1, 10-12). In this section by means of the
attactive elucidation and stereromeric studies of C-glucu-
ylsotetones of the stoe type and C-glycosylantrones of the
1H-hydroxyflavone-type, we show which methods are used
to the elucidation of such phenomena and which information

Effects can be obtained from them: X-ray crystallography, molecular
Aloesin, which is known to be present in at least 68 Aloe species, may be regarded as parent structure for other naturally occurring diasteremic 10-glucosylanthrones. Almost 140 years after the first isolation of aloesin from the world-wide used drug Aloe barbadensis (121), the absolute configuration of aloesin B at C-10 has now been established (8a) by an X-ray structure analysis as 10R, 1'S, and thus that of aloesin 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 "solenoid" 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 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).

Diasteremic oxoanthrone-C-glucosyls, in which C10 of the glucopyranosylanthrones is oxidized, have now been detected as a novel anthranoid principle from Cascara bark, namely the 10- hydroxyxalyl-type as well as their corresponding O- glucosides of the 10-hydroxycascorubide-type (8c, 102). The configurations of these stereoisomers were established by an X-ray structure analysis of 10-hydroxyxalyl A as 10R, 1'R, and it then followed, that the configuration of B is 10S, 1'R (8b).

Fig. 2: Determination of the absolute configuration of Aloe B by two-dimensional 1H NMR spectroscopy. The ROESY spectrum shows the repulsion between the glucosyl-2', 3', and the aromatic H-1 indicating a 10 R configuration.
Table 2: The absolute configurations of naturally occurring yaconose-10-C-3,5-dihydroxy glucose and 2H-13C NMR spectroscopic data (129) correlated to the X-ray analyses (8,9).

<table>
<thead>
<tr>
<th>Aloin A-Type</th>
<th>Configuration</th>
<th>Aloin B-Type</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>C-10</td>
<td>B</td>
<td>C-10</td>
</tr>
<tr>
<td>(1) Aloe A</td>
<td>S</td>
<td>(2) Aloe B</td>
<td>S</td>
</tr>
<tr>
<td>(3) Aloe A</td>
<td>S</td>
<td>(4) Aloe B</td>
<td>S</td>
</tr>
<tr>
<td>(5) 5-Oxycatin A (Chrysalis)</td>
<td>S</td>
<td>(6) 5-Oxycatin B</td>
<td>S</td>
</tr>
<tr>
<td>(7) Terratin A</td>
<td>S</td>
<td>(8) Terratin B</td>
<td>S</td>
</tr>
<tr>
<td>(9) 5-Oxycatin A</td>
<td>R</td>
<td>(10) 5-Oxycatin B</td>
<td>S</td>
</tr>
<tr>
<td>(11) 7-Hydroxy A</td>
<td>F</td>
<td>(12) 7-Hydroxy B</td>
<td>S</td>
</tr>
<tr>
<td>(13) B-Methyl-7-Oxycatin A</td>
<td>S</td>
<td>(14) B-Methyl-7-Oxycatin B</td>
<td>S</td>
</tr>
<tr>
<td>(15) Homomorin A</td>
<td>S</td>
<td>(16) Homomorin B</td>
<td>S</td>
</tr>
<tr>
<td>(17) Cascaroid A</td>
<td>S</td>
<td>(18) Cascaroid B</td>
<td>S</td>
</tr>
<tr>
<td>(19) Cascaroid C</td>
<td>R</td>
<td>(20) Cascaroid D</td>
<td>S</td>
</tr>
<tr>
<td>(21) Cascaroid E</td>
<td>R</td>
<td>(22) Cascaroid F</td>
<td>S</td>
</tr>
</tbody>
</table>

C-Glucoxybenzenes (R=OH)

<table>
<thead>
<tr>
<th>10-Hydroxy A-Type</th>
<th>Configuration</th>
<th>10-Hydroxy B-Type</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>C-10</td>
<td>B</td>
<td>C-10</td>
</tr>
<tr>
<td>(23) 10-Hydroxy A</td>
<td>R</td>
<td>(24) 10-Hydroxy B</td>
<td>S</td>
</tr>
<tr>
<td>(25) 10-C-Glucoxy-10-Hydroxy-2,6-dimethoxyphenyl A</td>
<td>R</td>
<td>(26) 10-C-Glucoxy-10-Hydroxy-2,6-dimethoxyphenyl B</td>
<td>S</td>
</tr>
<tr>
<td>(27) 10-Hydroxybenzoxyl A</td>
<td>R</td>
<td>(28) 10-Hydroxybenzoxyl B</td>
<td>S</td>
</tr>
<tr>
<td>(29) 10-Hydroxybenzoxyl E</td>
<td>S</td>
<td>(30) 10-Hydroxybenzoxyl F</td>
<td>R</td>
</tr>
</tbody>
</table>

In conclusion, the stereochemistry of the yaconose and its derivatives have been confirmed through various spectroscopic techniques, including 1H and 13C NMR, and X-ray crystallography. The absolute configurations of the 10-hydroxy derivatives were determined, confirming the enantiomeric purity of the natural products. The structural elucidation of these compounds is essential for understanding their biological activities.