Investigations on West African medicinal plants

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Abstract— We report on the isolation and structural determination
of and synthetic studies with constituents of the West African
medicinal plants Tabernaemontana glandulosa, Carissa edulis
(Apocynaceae), Canthium subcordatum (Rubiaceae), Hexalobus
crispiflorus (Annonaceae), Uvaria elliotiana (Annonaceae),
Annonidium manii (Annonaceae), Cochlospermum planchonii
(Cochlospermaceae) and Iboza riparia (Labiatae).

This report deals with investigations on West African medicinal plants. We
started this work about 8 years ago in cooperation with people from the
University of Ghana. Today I would like to report on some details of what we
have done with special emphasis on some of our most recent results.

Tabernaemontana glandulosa is a shrub which belongs to the Apocynaceae
family. Parts of the plant are used in folk medicine against various
diseases. From the stems and leaves we isolated as a main constituent
tabernusoline, a new alkaloid.

In pharmacological tests, tabernulosine causes a significant hypotensive
effect, when injected intravenously into genetic hypertonic rats (Fig. 1).
After a single dose, the blood pressure falls by 25 mm Hg, and remains at
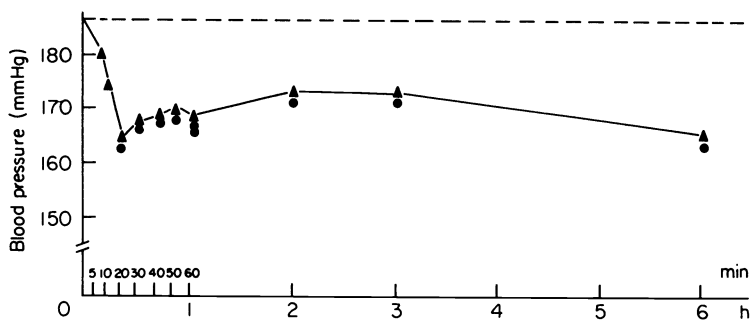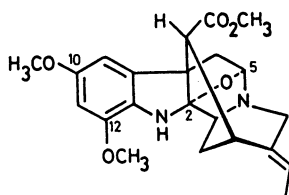this lower level for a considerable time.

Fig. 1 Influence of tabernulosine on blood pressure of genetic
hypertonic rats after administration of 18 mg/kg i.v.

Formula 1 was established for tabernulosine and this is the absolute
configuration, which was deduced by chiroptical measurements.

tabernulosine (1)

As one realizes, tabernulosine exhibits
the interesting ring system of the picrin-
ine alkaloids, of which, comparatively few
representatives are known. The hexacyclic
ring system is characterized by an oxygen
bridge between C-atoms 2 and 5, thus creat-
ing the structural element of an α,α'-di-
aminoether. This structural situation causes
unusual reactivity when exposed to
reductive agents and I will refer to that
point later. Let me make a remark con-
cerning the 10.12-disubstitution in the
benzene ring: similarly substituted
aromatic systems are very often encountered in natural products derived from
acetate, for example in flavonoids. But this type of substitution is very
unusual for alkaloids.

[13]C-nmr served to establish this substitution pattern unambiguously. As the
basic resonance values of the dihydroindole system we used for our
calculations the resonance assignments of the alkaloid andrangine, which has
been thoroughly studied by POTIER and coworkers (ref. 1). Time dones not
permit me to discuss the structure work in detail, but I would like to add a
few words on the electron collision induced fragmentation of tabernulosin and
this type of alkaloids in gneral: mass spectra show surprisingly few fragments.
An intense molecular ion, and the appearance of the base fragment at M-99mu
are fairly typical. On the basis of special studies on the mass spectroscopic
behaviour of tabernulosine and 12-demethoxy-tabernulosine we suggest
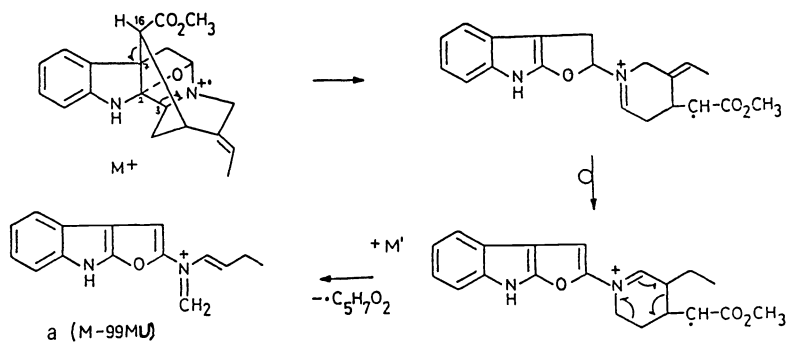structure a for the base fragment, and the route of formation in Figure 2.

Fig. 2 MS- fragmentation of picrinine-type alkaloids

Primary cleavage of the 2/3 and 7/16 bonds is induced by.the electron septet
on the alicyclic nitrogen. A rearranged molecular ion can then be produced
via an indolodihydrofuran by hydrogen transfer and shift of the double bond.
We propose the indolofuran structure M' for the rearranged molecular ion; if
C-16 carries a carbomethoxy function as in tabernulosine, 99 mass units can
then be removed as $C_5H_7O_2$ in the course of a retro-diene cleavage of the
piperideinium ring system, and the highly conjugated immonium ion a is
produced. Back to the reactivity of 1, Fig. 3 compiles some results how
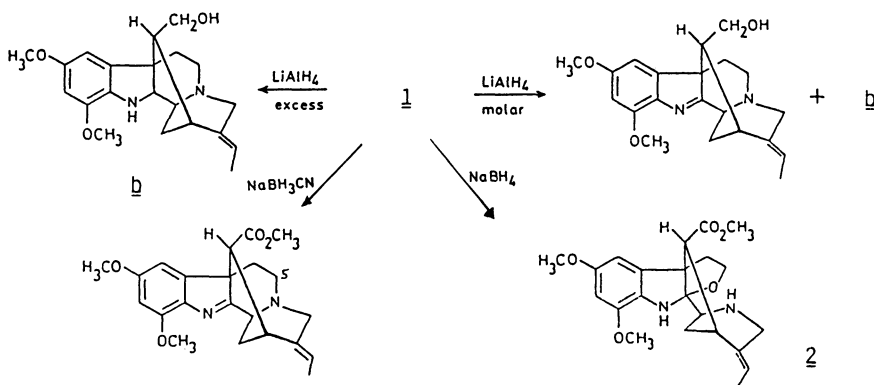tabernulosine reacts with well-known metal-hydrides.

Fig. 3 Reactivity of 1 with metalo-hydrides

Minimal change in the molecule is caused by sodium cyanoborohydride; the
oxygen bridge is removed and a 1/2 double bond is introduced with formal
transfer of hydrogen from N-1 to C-5. Lithium aluminium hydride in excess –
besides desoxygenation – reduces the imino double bond and of course the
ester group. In contrary to the desoxygenations, sodium borohydride breaks
the N-4/C-5 bond and produces the hydroindolo-tetrahydrofuran derivative 2.
Among the more than 15 minor alkaloids from T.glandulosa 19-hydroxy-coron-
aridine (3) should be mentioned. It also easily
can be prepared from coronaridine by oxidation
using iodine in the two phase system benzene/water.
(By the way, coronaridine itself could not be
detected in T.glandulosa).

3 possesses antibiotic activity and so far
deserves special attention in regard to its
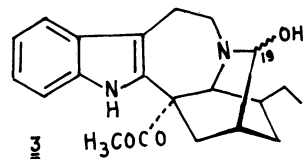effects on Gram-negative bacteria.

Table 1 presents some quantitative data and it is obvious that minimum inhibitory concentrations against various germs are pretty low. For example **Pseudomonas aeruginosa** belongs to the bacteria which are highly sensitive against 19-hydroxy-coronaridine. These results caused us to perform experiments on structure-activity relationships. We prepared and tested a number of structurally related alkaloids. The result is summarized in Fig. 4: for good antibacterial activity a hydroxyl at C-19 is essential; but amazingly, by removal of the carbomethoxy group from C-18 a considerable increase of the antibiotic effect is achieved. **Uvaria elliotiana** is another African medicinal plant; it belongs to the Annonaceae family.

TABLE 1 19-Hydroxy-coronaridine: antibiotic activity against various Gram-negative bacteria

Organism	MIC	Organism	MIC
Achromobacter geminianii	10 µg/ml	E. coli ton A von aro B	10 µg/ml
Aerobacter aerogenes	>100 µg/ml	Proteus vulgaris	100 µg/ml
Agrobacterium tumefaciens	0.01 µg/ml	Pseudomonas aeruginosa	0.01 µg/ml
Chromobacterium violaceum	>100 µg/ml	Pseudomonas fluorescens	>100 µg/ml
Escherichia coli ATCC 8739	100 µg/ml	Salmonella typhimurium	>100 µg/ml
E. coli Wildtyp aro B	10 µg/ml		

coronaridine

coronaridinelactam

not active   voacangine

voacanginelactam   very weakly active

19-hydroxy-coronaridine   <   19-hydroxy-ibogamine
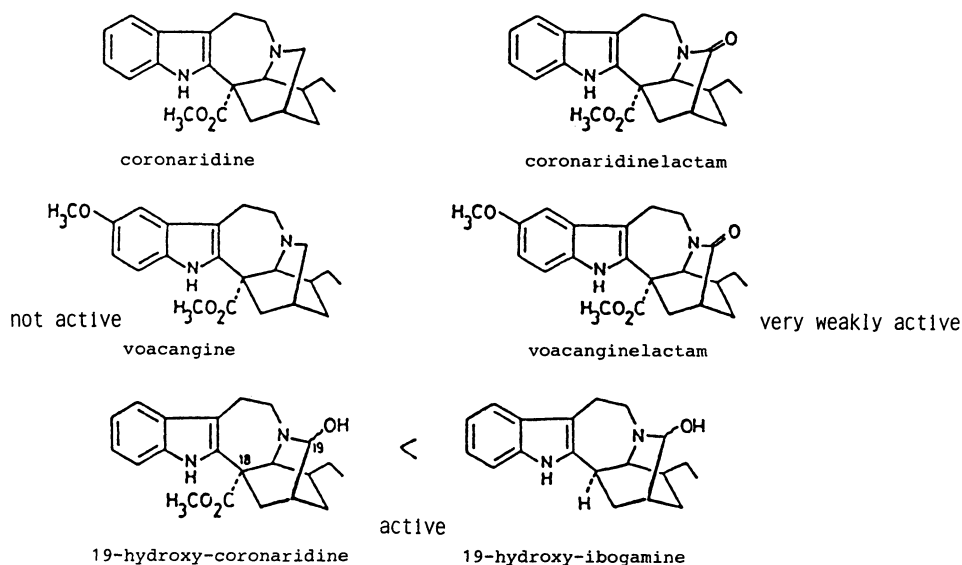   active

Fig. 4 Structure-activity relationship of antibiotic activity

The chief basic component of the bark was a new compound with a simple formula. Structural studies lead to 3,6-bis(3-methyl-2-butenyl)-indole (**4**). It is worth mentioning that this structurally new alkaloid possesses antibiotic properties against some fungi (e.g. **Mucor** **mihei**). **4** at the time of its detection was also interesting from the standpoint of chemotaxonomy, because it was isolated from an Annonaceae species and this family was known preferentially to contain alkaloids of the benzyl-isoquinoline-aporphine group.
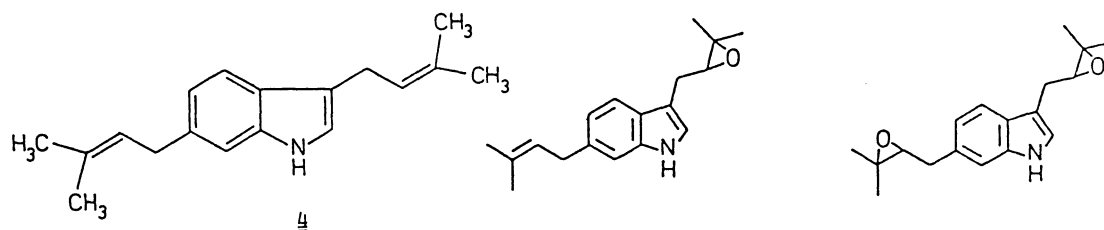
4

Fig. 5 Hexalobines from **Hexalobus crispiflorus** and from **H. Monopetalus**

Today we obviously have to regard alkaloids of the diprenylated indole type
as characteristic constituents of at least various members of the Annonaceae,
since meanwhile we detected 4 in two different Hexalobus species, which also
belong to the Annonaceae. But in contrary to Uvaria, in Hexalobus 4 was
accompanied by alkaloids of the noraporphine type and in addition by further
structurally related diprenylated indoles.

Up-to-now we know more than 15 individual alkaloids in that class. Therefore,
I would like to name this new group of natural products the hexalobines.
As Fig. 5 shows, structural variety basicly comes from oxidation of the
double bond(s) to epoxide(s) and these compounds were isolated from
H.crispiflorus as well as from H.monopetalus.

However, in H.crispiflorus structural variety was found particularly wide:
substitution pattern of the indole is not restricted to 3,6-, but also 3,5-
and even 2,3-diprenylated indoles occur and it exists a wide structural
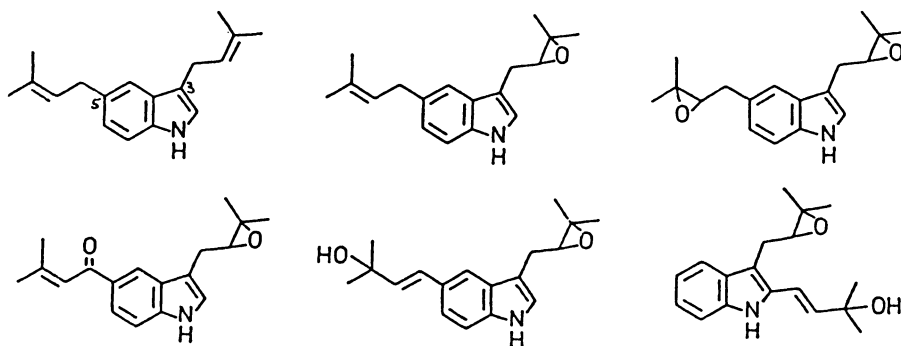variety within the side chains (Fig. 6).

Fig. 6 Further hexalobines from Hexalobus crispiflorus

In addition, from H.crispiflorus we isolated 6 esters of hexalobines with a
1,3-diolic structure at a rearranged isoprene system. As acidic components
of these esters palmitic, oleic and linoleic acid have been found (Fig. 7).
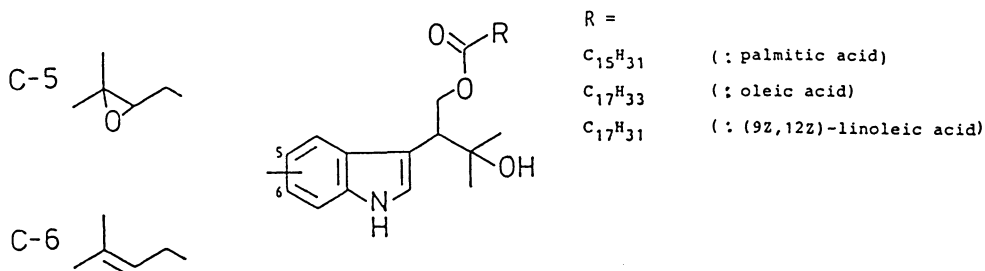

R =

$C_{15}H_{31}$ (: palmitic acid)

$C_{17}H_{33}$ (: oleic acid)

$C_{17}H_{31}$ (: (9Z,12Z)-linoleic acid)

Fig. 7 Esters in the hexolibine series

For our structure work in the hexalobine series and particularly to establish
the positions of the C_5-substituents at the indole nucleus, ^{13}C-nmr studies
were extremely helpful. Figure 8 gives an example:

C-2 :	124.1	C-5 :	121.7
C-3 :	102.1	C-6 :	119.6
C-3a:	127.6	C-7 :	111.0
C-4 :	120.5	C-7a:	135.5

indole   4

C-3 : 102.1 → 116.0

C-6 : 119.6 → 135.8

C-7 : 111.0 → 110.3 (dq, $^1J_{C-H}$= 157 Hz, $^3J_{C-H}$= 7 Hz)
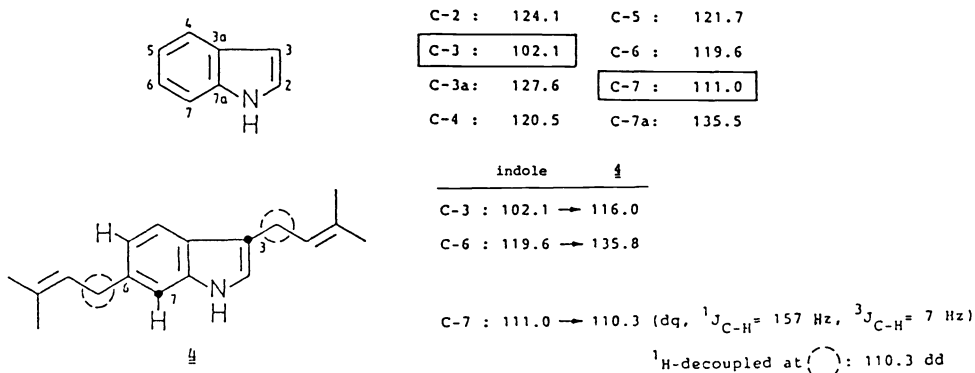
^{1}H-decoupled at ( ): 110.3 dd

4

Fig. 8 ^{13}C-nmr shift values

In unsubstituted indole the signals of C-3 and C-7 appear at highest field; the C-4 signal is found significantly lower. Alkylation causes characteristic downfield shifts; in this case from 102 to 116 ppm and from 119 to 135 ppm. To prove definitely that the substituent is a C-6 and not at C-5 can be confirmed by SFORD (=single-frequency-off resonance) experiments and observation of the long range proton/carbon splittings of C-7. The signal of C-7 appears as a double-quartet by long range coupling with H-5 and the methylene protons in the side chain. Irradiation at the -CH$_2$-proton frequency simplifies C-7 to a double-doublet.

As to the chemical reactivity in the hexalobine series I would like to draw your attention to the ring-opening reaction of the oxirane ring in β, γ - position to C-3 of indole, which on proton catalyzed hydrolysis gives two products: the expected 1,2-diol 5 and an isomeric 1,3-diol 6, which must originate from a rearrangement of the C$_5$-chain (Fig. 9).
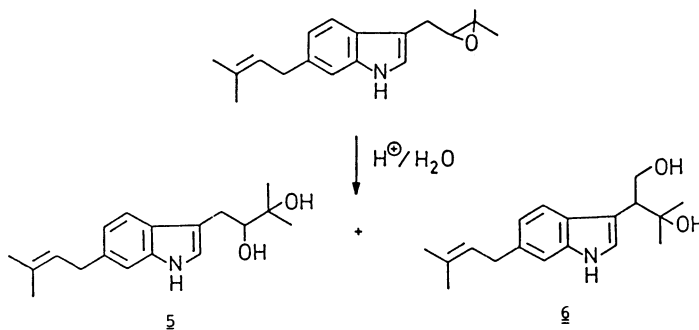

Fig. 9 Proton-catalyzed ring-opening reaction

The result can be explained by the formation of a cyclopropane intermediate, which takes place by interaction of the 2,3 double bond from indole like an ene-amine system.

Nucleophilic attack of water now can occur either to give the usual 1,2-diol 5 (Fig. 10, route A) or alternatively at the "upper" carbon atom which produces the rearranged 1,3-diol 6 (route B).
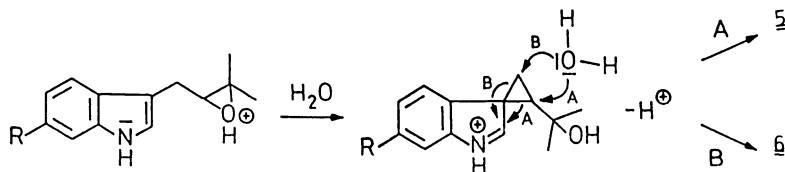

Fig. 10 Proposed mechanism of ring-opening reaction

Synthesis of the basic hexalobines was performed via the corresponding 3-methylbuta-1,3-dienyl substituted indoles.
In the 3,6-substitfuted series we started from 6-formyl indole and by WITTIG reaction primarily prepared an E/Z mixture of the diene 7 (Fig. 11).
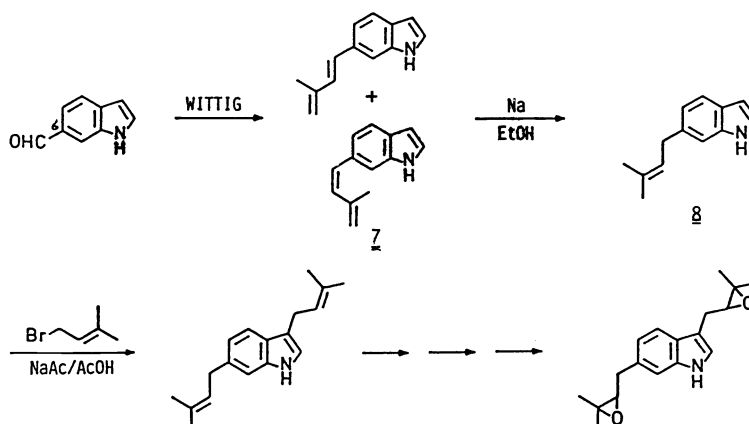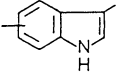

Fig. 11 Syntheses in the hexalobine series

Table 2 Antifungal activity against _Saprolegnia asterophor_

	C-5	—				
	C-6	—	+	+ +	+ +	—
	C-5	+	—			—
	C-6	+				
	C-5	+	—			

++: very active; +: active; -: not active

1,4-Hydrogenation of the diene system produces 6-(3-methyl-2-butenyl)-indole (8), which easily can be alkylated in the 3-position. I should mention that the E-isomer of 7 and the 6-mono-prenylated indole 8 as well are also genuine natural products (ref 2-3); both occasionally have been synthesized by Japanese and German groups (ref 4-6). Tosylation protects the indole nitrogen against attack of m-chlorobenzoic peracid in the next reaction step and this - depending on the amount of oxidizing agent - yields the tosylates of the di-epoxide and the two mono-epoxides. Chromatographic separation and detoxylation gives the natural procucts, but as racemates. In case of the di-epoxide the synthetic material is accompaned by its stereo-isomer, which exhibits almost identical physico-chemical properties.

In antifungal tests most of the hexalobines proved to be biologically active. The activity depends on the test organism. Against _Saprolegnia asterophor_ the epoxides were pretty active and highest activity was found in the diols (Table 2).

Besides the hexalobines, from the Hexalobus species we isolated quite a number of further alkaloids. The structures isolated from _Hexalobus crispiflours_ are shown in Fig. 12 and they are mostly of the noraporphine-type.

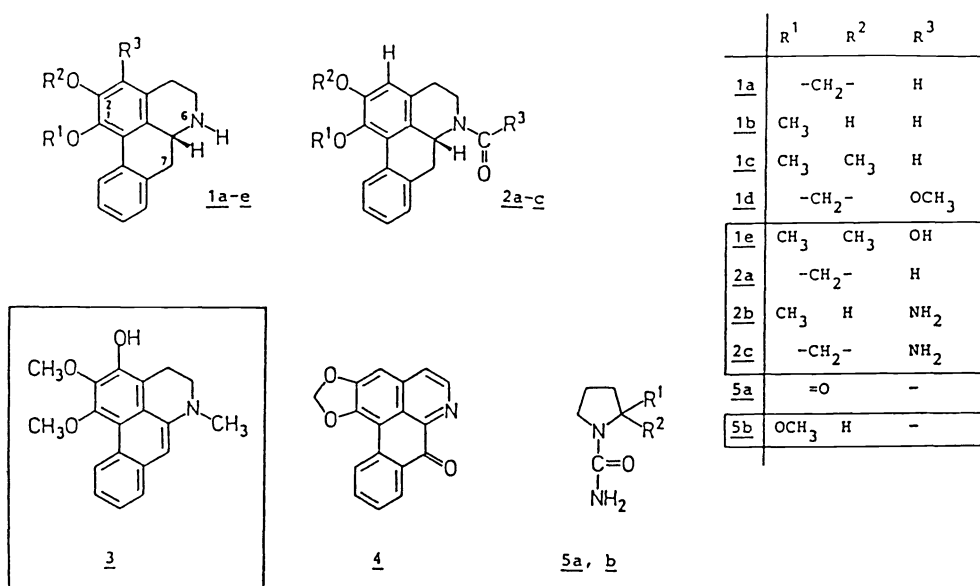|	R^1	R^2	R^3
1a	-CH$_2$-		H
1b	CH$_3$	H	H
1c	CH$_3$	CH$_3$	H
1d	-CH$_2$-		OCH$_3$
1e	CH$_3$	CH$_3$	OH
2a	-CH$_2$-		H
2b	CH$_3$	H	NH$_2$
2c	-CH$_2$-		NH$_2$
5a	=O		-
5b	OCH$_3$	H	-

Fig. 12 Further alkaloids from _Hexalobus crispiflorus_

Among these structures the framed ones are new alkaloids. Compounds 2b and
2c represent alkaloids, in which the nitrogen is part of an urea group.
It is worthwhile shortly to mention the unexpected reactivity of 5b.
Of course, 5b must not necessarily be a genuine natural product, but amazing
is its easy polymerisation in the presence of acids and the formation of
only one main product. This reaction occurs even in the chloroform solution
prepared for nmr measurements, if the chloroform used is not well
pretreated. Field desorption ms clearly demonstrates that a tetramerization
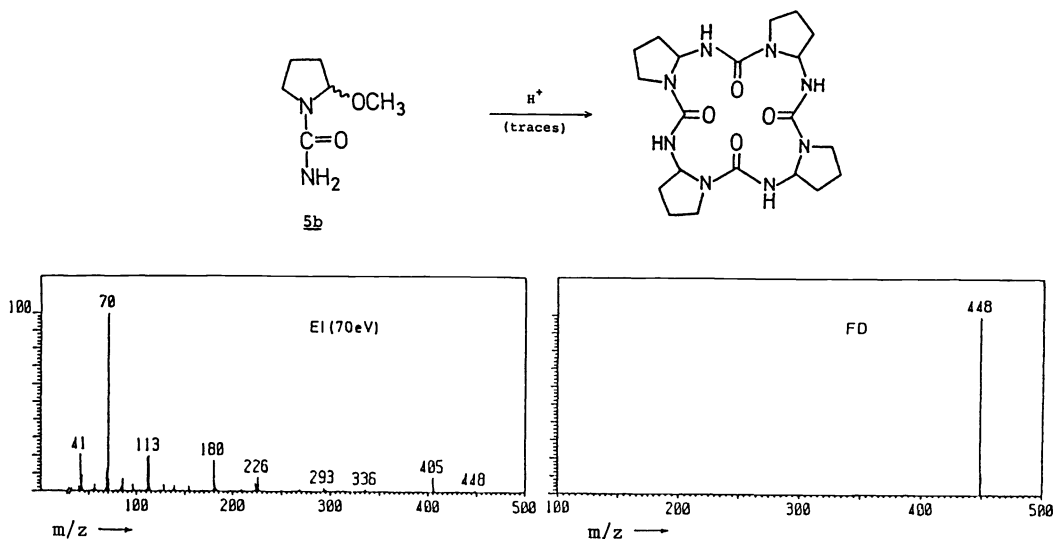takes place (Fig. 13).

Fig. 13 Tetramerization of N-carbamoyl-2-methoxy-pyrrolidine (5b);
EI- and FD-ms of product

Back to the structure elucidation of the 1,2,3-trisubstituted noraporphine;
we faced the problem to assign the substituents to their correct positions.
This is not an easy task, particularly if only small amounts of an alkaloid
are available and therefore sophisticated nmr studies can not be performed.
My statement is documented by reports from the newer literature, where final
structures of isolated alkaliids of this type could only be reduced to
alternative structural proposals (ref 7). However, in the course of our
structure work we found, that mass spectrometric investigations can be used
to unambiguously determine an oxygen substituent at C-3 in nor-aporphines: we
detected, that N-acetylation of nor-aporphines opens a new fragmentation
pathway, in which the substituent at C-3 specifically becomes involved:
Figure 14 shows in its upper part (ms a.) the ms of the isolated nor-
aporphine, which carries two methoxyl- and one OH-substituent at ring A
according to ^{1}H-nmr. Ms b.) is taken from the acetyl derivative: One
recognizes a dramatic change of the fragmentation behaviour and a significant
loss of acetoxy and acetic acid from the molecular ion. Therefore, in this
process the N-acetyl can not have been envoluted, but the acetylated OH-group
at ring A must have been split off.

Mass spectrum C.) in Fig. 14 was run from the nor-aporphine derivative with
the shown structure; it corroborates this conclusion and gives additional
information: After N-acetylation the methoxyl substituent at C-3 is easily
lost.

Obviously, in the mass spectra of N-acetyl nor-aporphines there exists a
mechanism, which causes the specific loss of any substituent at C-3 from the
molecular ion.

Thus the acetyl derivative (Fig. 14 - b.) must be N-acetyl-3-acetoxy-1,2-
dimethoxy-noraporphine and the alkaloid isolated from H.crispiflorus (Fig.
12) must be the hitherto unkown 1,2-dimethoxy-3-hydroxy-noraporphine (1e).
Our explanation for this useful fragmentation pathway is outlined in Fig. 15.

By a McLafferty mechanism primarily a hydrogen atom from C-7 is transfered
to the amide oxygen and simultaneously ring B is opened to yield the
rearranged molecular ion M^1 with a fully conjugated phenanthrene moiety.
Subsequently the nitrogen displaces any substituent present at C-3;
consecutive loss of a hydrogen radical froms an ionic species stabilized by

extensive delocalization of the radical electron. Breakage of the C-4/C-5 bond in M[1] leads to the base fragment in the mass spectrum, the latter process is accompanied by loss of ketene, if R[3] is acetyl.

In conclusion, these studies establish a fast and easy mass spectrometric method to determine substituents at C-3 in alkaloids of the nor-aporphine-type.
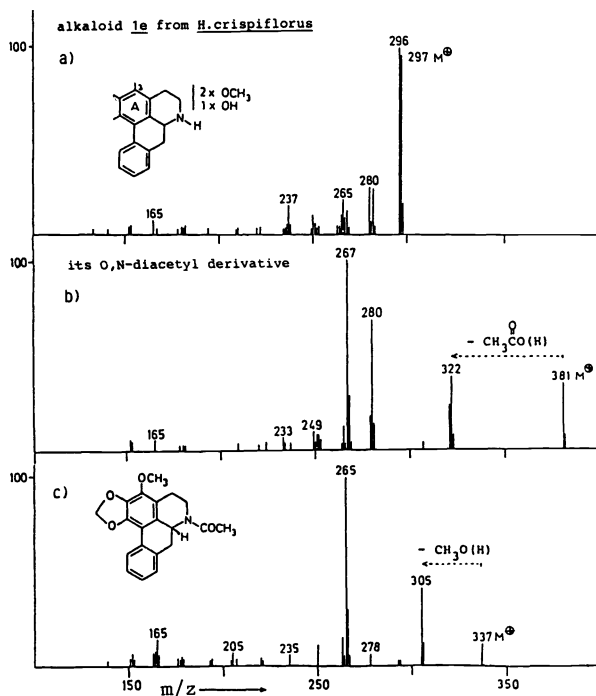

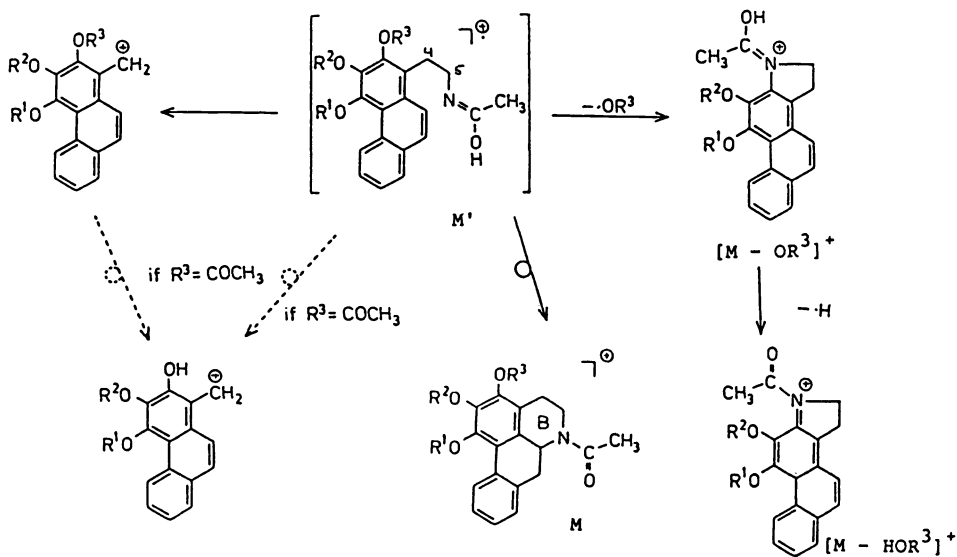Fig. 14 Mass spectra (EI) of 1e, its diacetyl derivative, and another N-acetyl-noraporphine

Fig. 15 Ms-fragmentation of 3-substituted N-acetyl-noraporphines; formation of key fragments [M − OR³]⁺ and [M − HOR³]⁺

Ammonidium mannii is another member of the Annonaceae family. The tree growths in West and Central Africa and its stem bark is used in folk medicine. From Annonidium mannii we isolated a number of new bisindoles, whose structures are closely related to the hexalobines (Fig. 16).

These annonidines can be regarded as biosynthetic dimerisation products between 7-, 6-, or 3-(3-methyl-2-butenyl)-indole, and I should add, that 7-(3-methyl-2-butenyl)-indole has also been found in the plant extract. The two indole nuclei are linked either via 1 carbon atom (1,1-annonidines) or via a C_3-bridge (1,3-annonidines).

These prenylated bisindoles, of the annonidine type - in contrary to many hexalobines - seem not to exhibit any antibiotic activity.
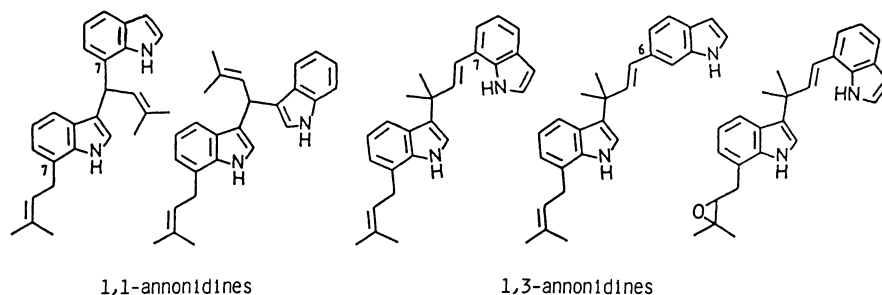

1,1-annonidines   1,3-annonidines

Fig. 16 The annonidines from Annonidium mannii

Extracts of the bark of Canthium subcordatum are used in African folk medicine for the treatment of high blood pressure. We therefore took an interest in the constituents of this member of the Rubiaceae family, and found, first of all, that the extracts except plain indole contain no alkaloids. The constituents are summarized in Fig. 17. Main component was the iridoid glucoside shanziside methylester (9) recently described by INOUYE as a minor component of Mussaenda species (ref 8); it was found to be inactive in pharmacological tests.

roseoside (0.1%)

9 (~5 %)

mannitol (2%); indole (0.5%)

orcinol monomethylether (0.6%)

scopoletol (0.8%)
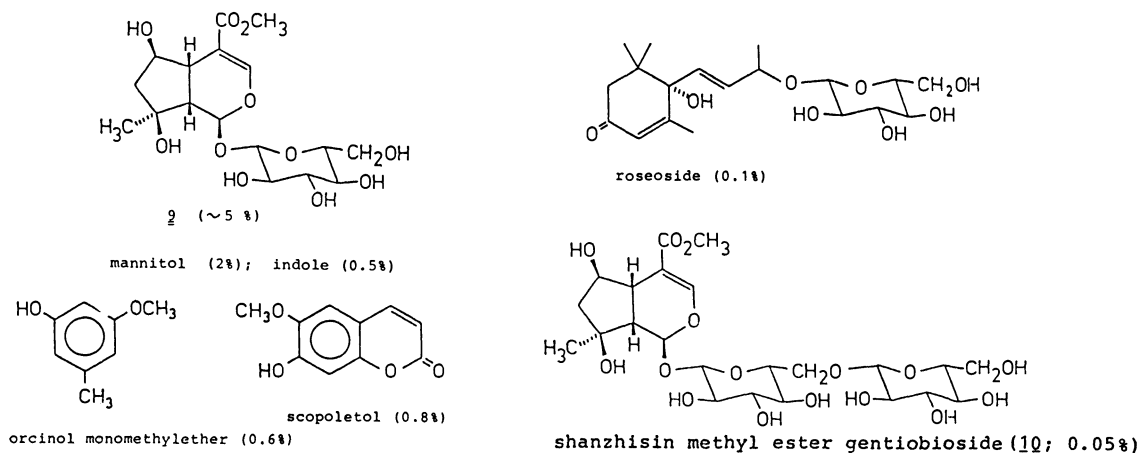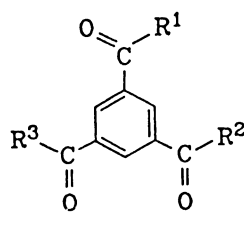
shanzhisin methyl ester gentiobioside (10; 0.05%)

Fig. 17 Compounds isolated from the root bark of C.subcordatum

In addition, mannitol, indole, orcinol and scopoletol were isolated, and roseoside, the rare glucoside of vomifoliol is also present. A further constituent was shown to have structure 10, which is a new iridoid with a disaccharidic sugar residue and should be named shanzhisin methyl ester gentiobioside. Since 10 occurs in the plant extract only in relatively small quantities, it was synthesized from the main iridoid (ref 9).

The last plants I would like to report on are cochlospermum planchonii and C.tinctorium, which belong to the comparatively small cochlospermaceae family. I want to mention the main constituents of the non-polar fraction in spite of the fact, that these probably are biologically inactive.

Figure 18 shows the structures of cochlospermines A to D: these are symmetrically substituted triacylated benzenes and they structurally differ only in the length of the acyl substituents and this only to a pretty small extent; of course these homologous substances had to be separated by high pressure liquid chromatography.

Compound	Elemental composition	$M^{\oplus}$	Primary McLafferty fragment(s) (m/z)	R^1	R^2	R^3
A	$C_{42}H_{72}O_3$	624	484	$H_3C[CH_2]_{10}$	$H_3C[CH_2]_{10}$	$H_3C[CH_2]_{10}$
B	$C_{44}H_{76}O_3$	652	512 and 484	$H_3C[CH_2]_{12}$	$H_3C[CH_2]_{10}$	$H_3C[CH_2]_{10}$
C	$C_{46}H_{80}O_3$	680	540 and 512	$H_3C[CH_2]_{12}$	$H_3C[CH_2]_{12}$	$H_3C[CH_2]_{10}$
D	$C_{48}H_{84}O_3$	708	540	$H_3C[CH_2]_{12}$	$H_3C[CH_2]_{12}$	$H_3C[CH_2]_{12}$

Fig. 18 **The cochlospermines from** Cochlospermum planchonii

The structure elucidation represents an interesting problem as far as the lengths of the individual substituents had to be determined without degradation.

Nmr can not answer this question because of the large size of the substituents. Again mass spectrometry gave the answer: induced by the carbonyl groups key fragmentation takes place between β-and γ-carbons of the side chains (by McLafferty rearrangement) and this occurs successively at the individual acyl groups (Fig. 19).
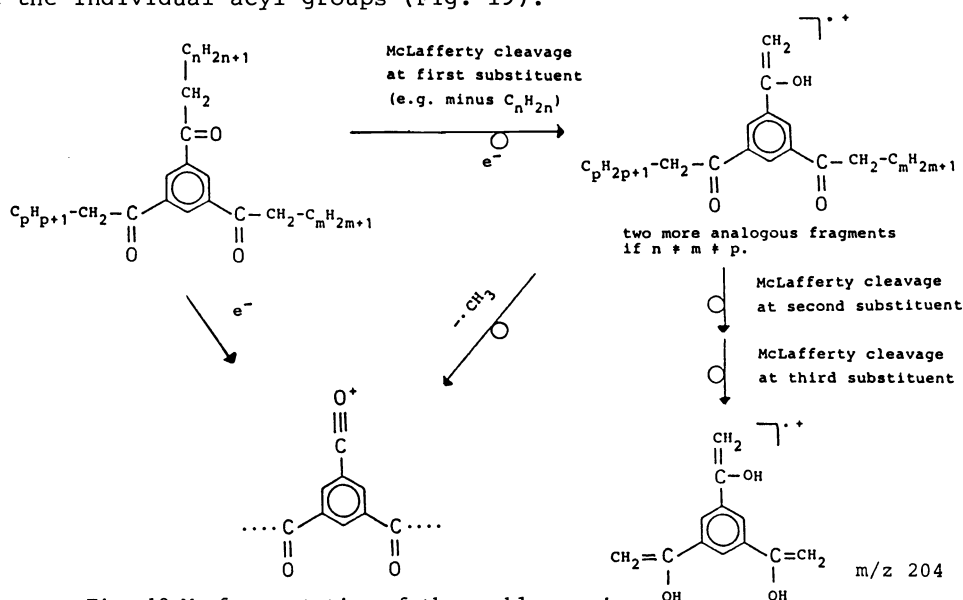

Fig. 19 Ms-fragmentation of the cochlospermines

Therefore, in the ms of cochlospermine A, which is the main component, three key fragments occur by regular and successive loss of $C_{10}H_{20}$ each, whereas the ms of cochlospermine B exhibits five key fragments: Primary fragmentation of cochlospermine B occurs by alternative loss of $C_{10}H_{20}$ or $C_{12}H_{24}$ from the M and therefore produces primary fragments at m/z 512 and 484.

That is the end of my report on some structural and biological aspects of our recent research on West African medicinal plants. I occasionally went more into detail as far as structure elucidation was concerned. I only shortly mentioned part of our synthetic work.

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