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Analytical Studies on the Structure of Natural Products from Menispermaceae Drugs

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Lianas from the family Menispermaceae, which are spread in the tropics and subtropics all over the world, are very important for the traditional medicine of the indigenous people. The role of these drugs can be explained at least partially by their ingredients. Till now the most investigated ingredients are alkaloids which are derived from the benzylisoquinoline group. The as well typical bitter tasting isoprenoids are hardly inverstigated especially if they are glycosides. Folk medicinal indications of Menispermaceae drugs are all over the world nearly the same, for example:

fever,
cough,
jaundice,
cholera,
gastro intestinal diseases,
rheumatism,
veneral diseases,
snake bite and last but not least: for a long Life

Our first work with Menispermaceae drugs

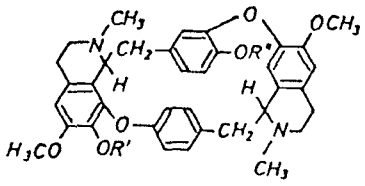
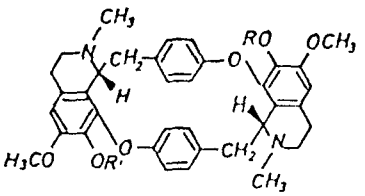
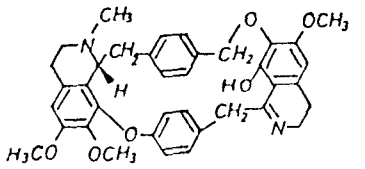
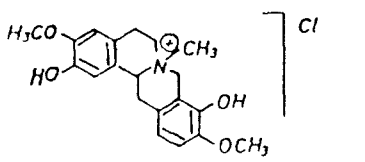
		R' R"	*Fundort*
	*l-(−, −)-Curin*	H H	Indien
	Chondodendrin, Bebeerin, Pelosin, Cissampelin		S-America
	Hayatin	H H	Indien
	d,l (+ + −−)-Chondodendrin		
	Hayatinin		
	d,l-O-Methyl-chondodendrin		
	Hayatidin		
	(+ −)-O-Methyl-chondodendrin-bebeerin-curin	H CH_3	Indien
	(+ +)-*O-Methyl-curin* -chondodendrin	H CH_3	Jamaika
	*d-Isochondodendrin,* Isobebeerin	H H	Indien
	Cycleanin	CH_3 CH_3	
	O-Methyl-isochondodendrin		Indien
	*Cissamparein*		Peru
	*Cissamin* = Cyclanolinchlorid		Indien
	Pareirin		Indien
	Menismin		Indien

Fig. 1. *Alkaloide aus Cissampelos pareira L.*

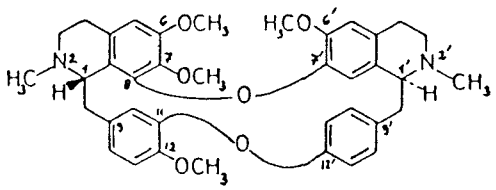
started nearly 20 years ago with the Thai drug "Krung Kha Mao" which botanical name was said to be "*Cissampelos pareira*". This plant is spread all over the tropical world and her ingredients are the relatively symmetrical bisbenzyl-isoquinoline alkaloids which are connected head to tail as shown in Figure 1.

Cissampelos pareira from Thailand was unexplored at that time. Therefore we started our research work with the root of this plant. Surprisingly we isolated as main alkaloid the head to head and tail to tail connected tetrandrine, a berbamine alkaloid which till now was found only as Figure 2, Ingredient of the related genus Cyclea[1]. Tetrandrine and the series of similar berbamine type side alkaloids let us suppose that the botanical name of Krung Kha Mao was really *Cyclea barbata* Miers. This was later on confirmed by botanical specialists from the Royal Botanical Garden in Kew/London.

In the early seventies our analytical and especially spectroscopic possibilities were very small and in the 60 MHz Proton NMR spectra the only detectable signals were these from methyl groups. These methyl signals gave in relation to the known data in the literature a relatively good hint for the structure together with the MS spectrum. These rather old result are represented again because they demonstrate very good the impressive advance of the analytical possibilities since that time.

Hint for the unsupposed tetrandrine were not only the physical data shown here but also the MS-fragmentation pattern which is typical for the bisbenzyl-isoquinoline alkaloids (Fig. 3).

Berbamine-type alkaloids as tetrandrine give a typical fragmentation of both benzylic tail parts and result the ion m/z 396 and especially the double charged fragment m/z 198. By lost of ring C and D originate the ion m/z 431 and by cleavage of ring E the ion m/z 485. Symmetrical head to tail connected bisbenzyl-isoquinoline alkaloids as curine in the MS only show a typical cleavage in two halves and no double charged ions.



S,S-Tetrandrin $[\alpha]_D^{20} + 282°$

^{1}H-NMR ppm
2-N-CH_3 2.30
2'-N-CH_3 2.60
7-OCH_3 3.18
6'-OCH_3 3.35
6-OCH_3 3.73
12-OCH_3 3.90

Fig. 2.

An important degradation for such alkaloids is the reductive ether cleavage found in the thirties from Sartoretto and Sowa[2] (Fig. 4.).

We have made at that time this ether cleavage with sodium in liquid ammonia and got as supposed from tetrandrine S-N-methyl-coclaurine, which was changed by O-methylation into the second cleavage product S-O-methyl-armepavine. This product was identic with the O-methylation product of authentic S-armepavine. Although the same result would given as well from S,S-dimethyl-curine, it was quite clear that we had found S,S-tetrandrine because of its characteristic MS fragmentation (Fig. 5).

At macrocyclic berbamine-type alkaloids the 6'-O-methyl signal in the proton NMR give a hint at the configuration of the chirality centres at C-1 and C-1'. From the total 14 isolated alkaloids found in Krung Kha Mao[3,4] the most were known from other plants. In figure 5 some examples with their methyl-signals are shown: Alkaloids with R,S -or S,R-configuration have deshielded their 6'-O -methyl-signal characteristic at lower field near 3.6 up to 3.8 ppm, while alkaloids with S,S- or R,R -configuration as tetrandrine show this 6'-O-methyl-signal at 3.4 ppm. In connection with the specific rotation one gets a sure hint at the absolute configuration of these alkaloids.

Two new compounds from *Cyclea barbata* I still like to mention here: A high polar alkaloid IVα[5] has an IR-spectrum quite similar to that from tetrandrine. The proton-NMR shows 4 O-methyl-signals as like as tetrandrine and additional at 3.55ppm a broad signal which related to 3 protons. From the both N-methyl-signals of tetrandrine that at lower field which indicates a 2'-methyl-group was absent.

The MS of IV α was except an additional signal at m/z 638 absolutely identic with that of tetrandrine (Fig. 6).

Indeed IV α was proved as 2'-N-oxide of tetrandrine which 2'-N-methyl-signal by the N-oxidation was deshielded. At that time N-oxides of bisbenzyl -isoquinoline alkaloids were unknown, therefore we proved that IVα was not produced artificially by the working up. Laurepukine, which was said to be an brenzcatechine-derivative, was really a pukateine-N-oxide shown by Weiss and Bernauer at 1971[6]. The N-methyl-signal of laurepukine was deshielded similar as the 2'-N-methyl-signal from

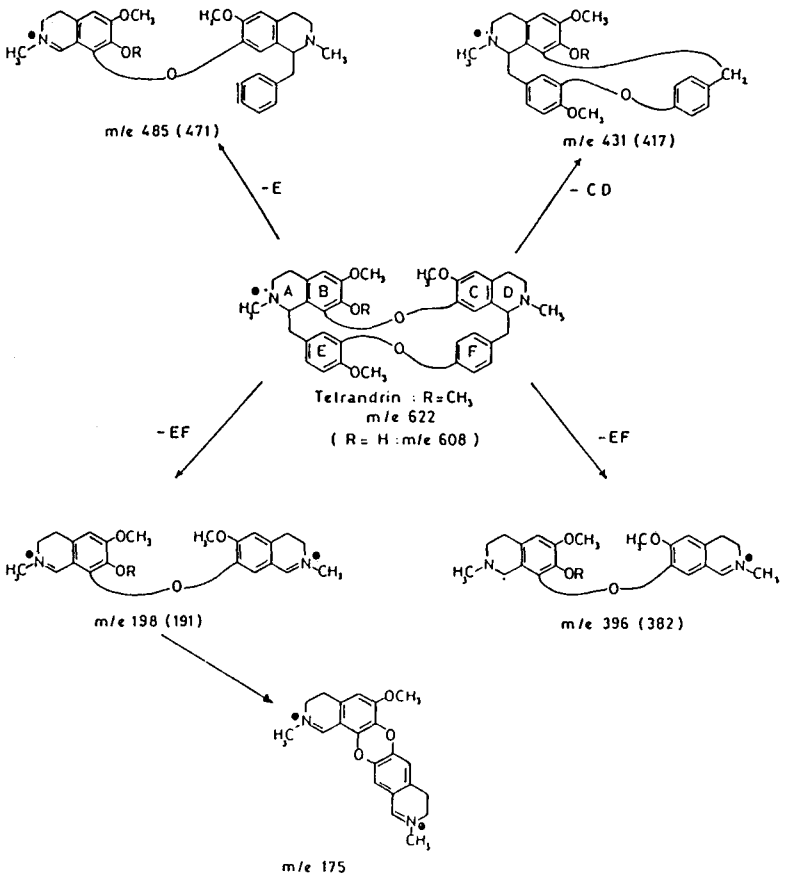

m/e 485 (471)
m/e 431 (417)
-E
-CD
Tetrandrin : R=CH3
m/e 622
(R= H :m/e 608)
-EF
-EF
m/e 198 (191)
m/e 396 (382)
m/e 175

Fig. 3.

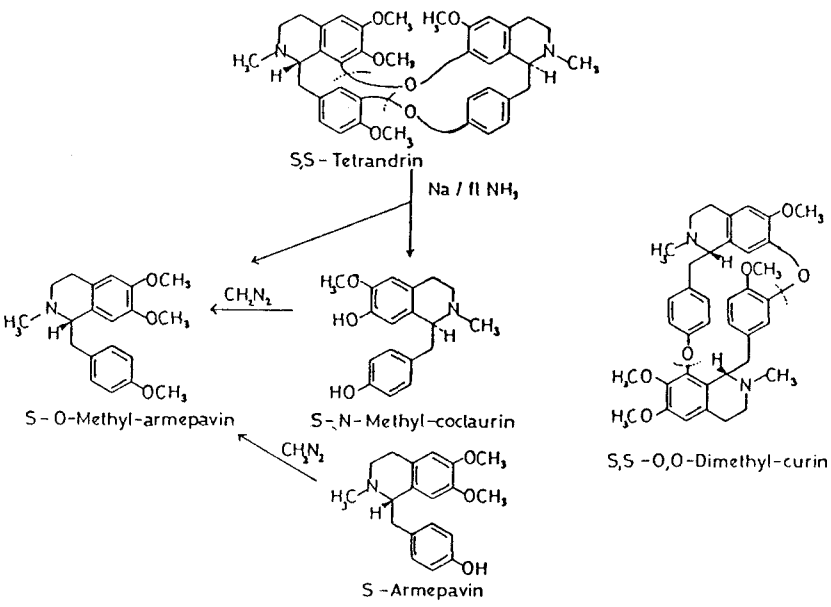
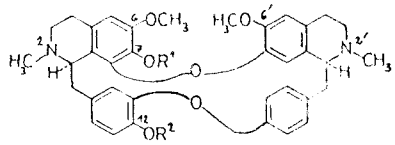

S,S - Tetrandrin
Na / fl NH3
CH2N2
S - O-Methyl-armepavin
S - N- Methyl-coclaurin
CH2N2
S - Armepavin
S,S - O,O-Dimethyl-curin

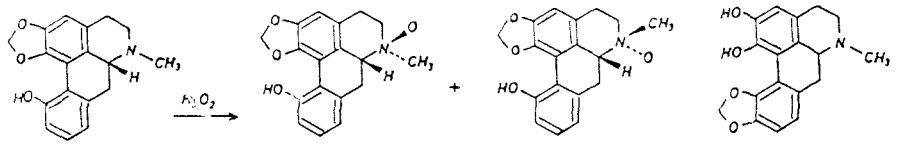
Fig. 4.



H-NMR- Signallagen (δ):

	R,S Berbamin $R^1 = CH_3$ $R^2 = H$	R,S Isofangchinolin $R^1 = H$ $R^2 = CH_3$	R,S Isotetrandrin $R^1 = R^2 = CH_3$	S,S Tetrandrin $R^1 = R^2 = CH_3$
2' N-CH_3	2.63	2.52	2.60	2.61
2 N-CH_3	2.27	2.32	2.28	2.31
12 OCH_3	—	3.93	3.95	3.91
6 OCH_3	3.81	3.92	3.78	3.73
6' OCH_3	3.64	3.78	3.63	3.36
7 OCH_3	3.17	—	3.18	3.19

Fig. 5.

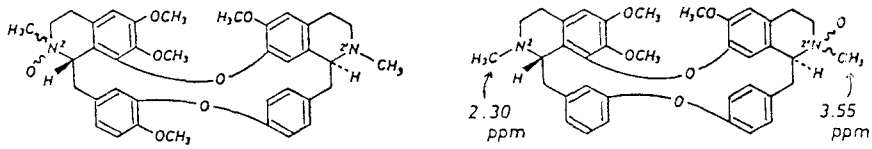


(-) Pukatein
N-CH_3: 3.37 ppm
(in CF_3COOH)

(-) Laurepukin
N-CH_3: 3.86 ppm

(-) Epi-laurepukin
N-CH_3: 3.86 ppm

Laurepukin
bisherige Formel
Weiss/Bernauer 1971



IVα Schmp. 192-193
$[\alpha]_D^{20}$ + 198° ($CHCl_3$)

Fig. 6.

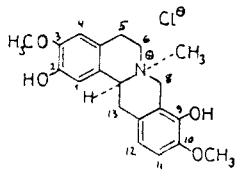
IVα . During MS-fragmentation N-oxides at first lose their N-oxide oxygen, then the N-oxide show the fragmentation pattern of the reduced compound. With 30% Hydrogen-peroxide one gets from tetrandrine a mixture of N-oxides, from which we isolated a 2'-N-oxide pair which has one identical set of proton NMR signals with that of IV α [5]. The MS from the 2'-N-oxide pair is absolutely identic with the MS from IV α . Since that time also from other plants natural bisbenzyl-isoquinoline-N-oxides are described (Fig. 7).

From the fraction of the quaternary alkaloids we isolated 2 tetrahydroberberine alkaloids which were identified as α-cyclanoline and the until now unknown β-cyclanoline and which are different at the configuration of the quaternary N-atom[7]. Both compounds were isolated from the unsoluble iodides mixtures and changed into chlorides as crystalline substance. IR and MS spoke undoubted in favour of the known α-cyclanoline, but the melting point and the specific rotation was quite different from the literature data. After reaction with diazomethane we yielded surprisingly not only the awaited O,O-dimethyl-derivative but a mono -methyl product too, which gave a positive Gibbs colour reaction. This gave a hint at a hydroxyl-group which is stereochemical hindered. The separation of α- and β-cyclanoline was successful realized chromatographically with neutral aluminium-oxide with methanol/water as eluant. Then

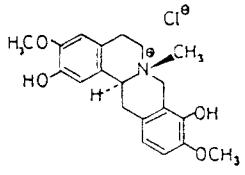
we were able to show that β-cyclanoline reacts very fast with diazomethane to the known β-dimethyl-steponine, while the stereochemical hindered α-cyclanoline does not react under these conditions. α-Cyclanoline reacts only with methyliodide and yield then the known α-dimethyl-steponine.

Though the pharmacological tests which we initiated from the crude extract and from tetrandrine too yielded no antipyretic activities, other people for tetrandrine (isolated from other species of the Menispermaceae family) have found remarkable antipyretic, antibiotic and especially antitumor activities. Therefore tetrandrine was clinical tested as cytostaticum[8]. But also other bisbenzyl-isoquinoline alkaloids have shown remarkable cytostatic and antibiotic properties (Fig. 8).

Macrocyclus and configuration of the chirality centre are important factor for the antitumor ac-



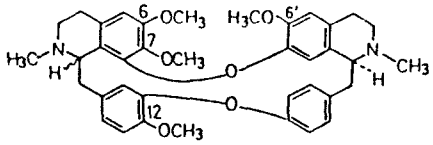
α-Cyclanolin-chlorid



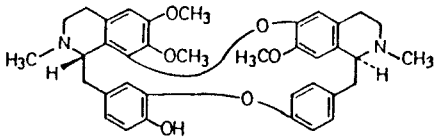
β-Cyclanolin-chlorid

Fig. 7.

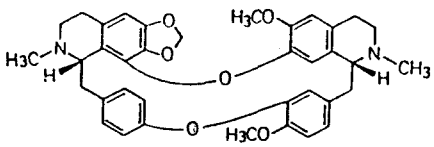
in vivo wirksam:



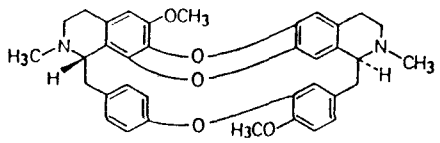
Tetrandrin (S,S)
Isotetrandrin (R,S)



Thalicberin (S,S)

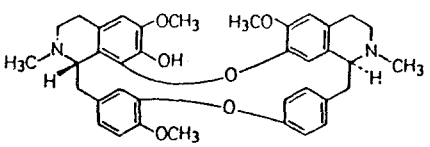


Cepharanthin (S,R)

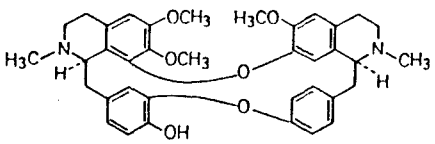


Isotrilobin (S,S)

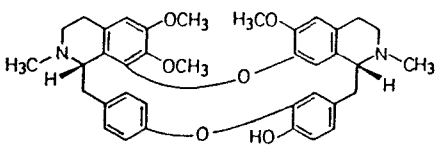
in vitro ± wirksam:



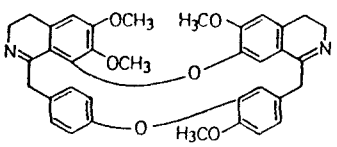
Fangchinolin (S,S)



Berbamin (R,S)



Oxyacanthin (S,R)



Stebisimin

Fig. 8.

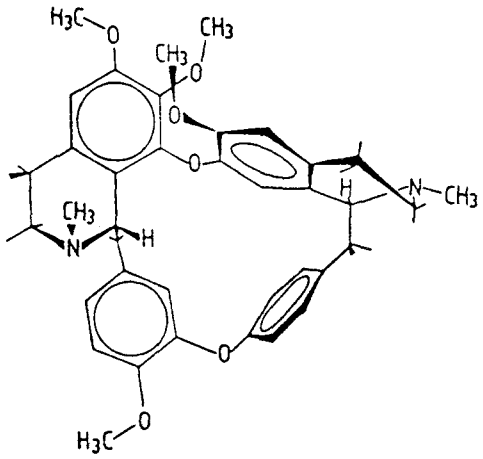


Fig. 9.

tivity[9]. The column of the left side of figure 8 shows alkaloids which are found active *in vitro* while the compounds of the right column only show a weak *in vitro* activity. The more active compounds have the 1-S, 1'-S configuration. The influence of number and position of the phenolic OH-groups at the cytostatic activity is almost unknown, evidently because suitable alkaloids with one or more phenolic OH-groups are not available in sufficient quantity. There we got enough tetrandrine from Krung Kha Mao we tried to prepare phenolic derivatives from tetrandrine by selective ether cleavage hopeful that we get enough material for a pharmacological screening. Only a few of the 15 possible ether cleavage products from tetrandrine are known as natural products (Fig. 9).

Here is shown the stereochemical correct formula of tetrandrine as found from M.S. Kupchan by the x-ray diffraction method. It seems to be possible that steric and electronic effects influence the result of an ether cleavage. The most interesting result of all possible cleavage methods we get with hydrobromic acid, which allowed a moderate selectivity of the results (Fig. 10).

One gets fangchinoline with nearly 35% beside 20% starting material and a trace of 3% of the diphenol **2b** by 1-day standing at room temperature with 62% hydrobromic acid. Ever 35% of atherospermolin **2a** and the 7,6',12-triphenol **3** one gets by 75 minutes at 100 °C with 47% hydrobromic acid.

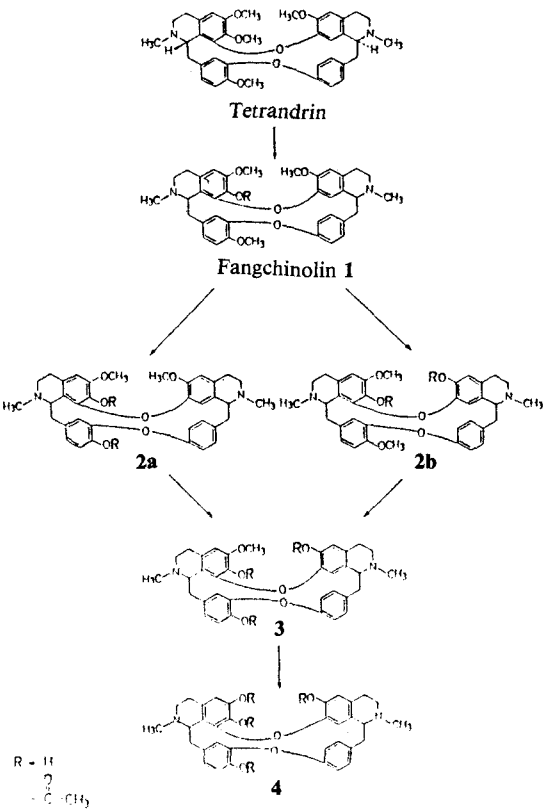
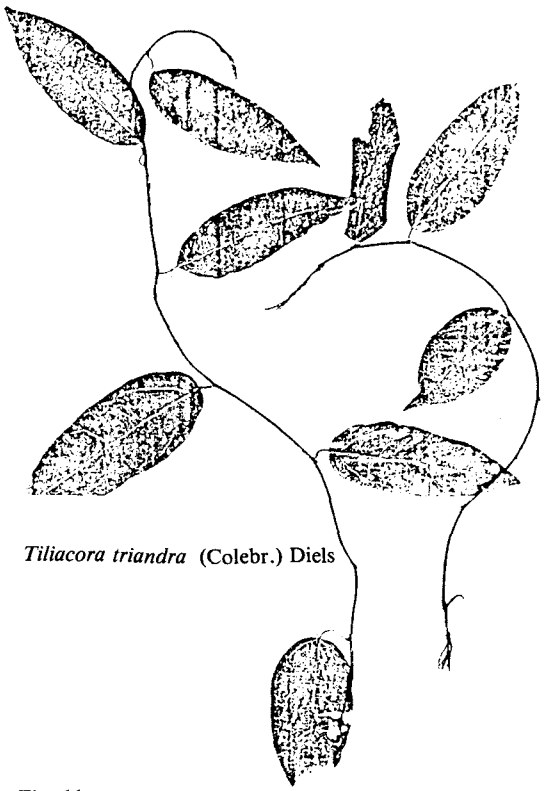


Fig. 10.



Tiliacora triandra (Colebr.) Diels

Fig. 11.

Nearly 100% of the tetraphenol **4** one gets after 2.5h at 100 °C with 62% hydrobromic acid. The separation of the reaction mixture is possible chromatographically with Sephadex LH 20 and chloroform/ethanol mixtures as eluant. So we were able to get fangchinoline, atherospermoline and the triphenol resp. the tetraphenol as well from tetrandrine with moderate expense[10]. Further investigations in this directions are in progress.

In Thailand the Menispermaceae drug "Yanang" which is *Tiliacora triandra* Diels, is used as medicine against fever, ...and so on. This plant is shown in Figure 11.

As at the other Menispermaceae one awaits alkaloids as ingredients of "Yanang", which are really found in the extract from stems and leaves of this plant (Fig. 12).

Just when we started our work with "Yanang" Wiriyachitra and coworkers[11] published 1981 that they have isolated and identified four bisbenzyl-isoquinoline alkaloids from the roots of *Tiliacora triandra,* namely tiliacorinine, nortiliacorinine A, tiliacorine and the new tiliacorinine-2'-N-oxide. The here shown TLC of our crude alkaloid fraction shows a lot of alkaloids, from which at least 10 are detectable by a blue colour with sulphuric acid containing 1% nitric acid. This colour reaction is a typical one for compounds with dibenzo-dioxine partial structure as found with tiliacora alkaloids. Additional these tiliacora alkaloids belonged to a relatively seldom found type of bisbenzyl-isoquinoline alkaloids which have both tail parts of the biogenetic coclaurine units connected by a C-C bond resulting there a diphenyl system.

DC vom rohen Alkaloidgemisch:

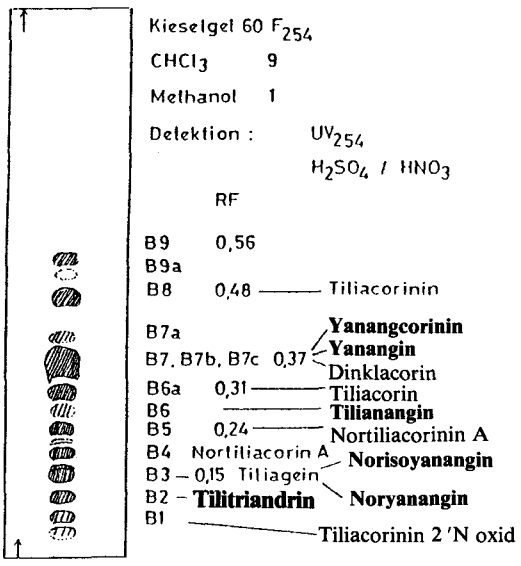


Fig. 12.

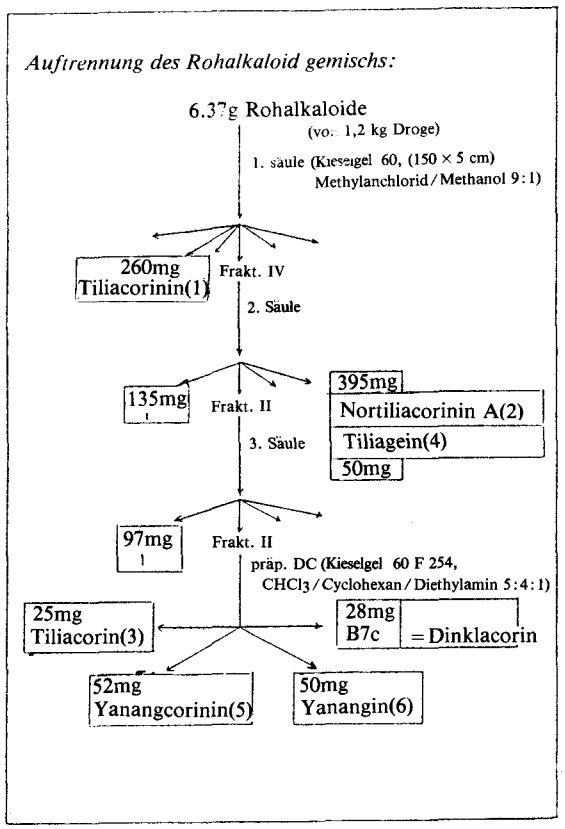


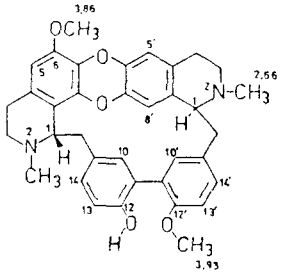
Fig. 13.

From the crude alkaloids mixture (5.3g/kg drug) we could isolate some new (underlined) alkaloids in amounts between 20 and 50 mg by low pressure column chromatography and preparative TLC (Fig. 13).

This isolation scheme shows the timly most expensive and less noticed part of a work on natural products. Additional to this simple scheme are typical difficulties as small amounts of single fractions, difficult and high-loss-inducing separation of side products, possible destruction and arteficial products. Even modern separation methods as HPLC or DCCC leave enough of these difficulties. At least every new isolated compound might be a known up to the last experiment.

From 6.4g crude alkaloid mixture in this way we got nearly 500 mg tiliacorinine and 400 mg nortiliacorinine A while the side products were separated in amounts between 10 to 50 mg by preparative TLC.

Here the formula is shown from yanangcori-



Yanangcorinin (**5**)
1S, 1'S
$[\alpha]_D^{20}$ + 368° ($CHCl_3$)
Molgew: 576 (MS)

^{1}H NMR (δ= ppm)	**5**	Tiliacorinin	CH_2N_2 → **5a** ≡ **1a**
2-NCH_3	2.33	3.28	2.30
2'-NCH_3	2.66	2.68	2.59
6-OCH_3	3.86	3.82	3.80
12-OCH_3	—	3.95	3.86
12'-OCH_3	3.93	—	3.88

^{1}HNMR (90 MHz) Tiliacorinin zum Vergleich

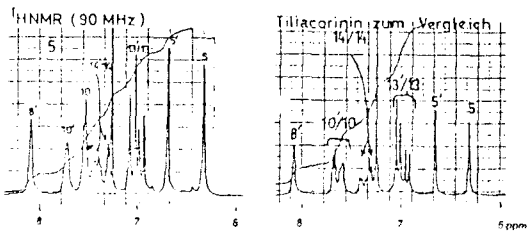
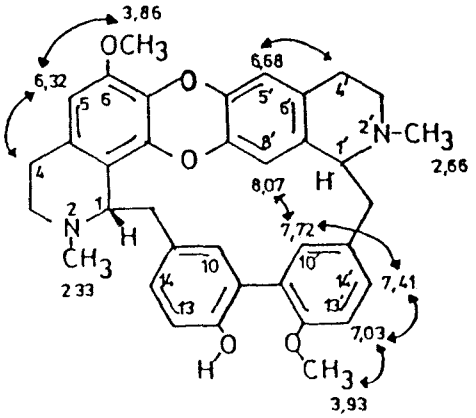


Fig. 14.

nine, a new alkaloid[12] which is quite similar to the main alkaloid tiliacorinine. The specific rotation hint at the S,S-configuration. The high resolved MS shows no difference between the MS of tiliacorinine. Both substances have practically identic IR spectra and the signals in the proton NMR shows so small differences, that one would believe that both substances are identic if only the NMR values are considered. Therefore it was quite probable that yanangcorinine was an isomer of tiliacorinine. In this direction hint also the specific rotation, which fit with the +316° up to +344° of tiliacorinine relatively good. The comparison of the aromatic proton-NMR-signals demonstrates that there is the same substitution pattern-very small differences in the region of the signals from H-13/H-13'hint at an isomerism in this part of the molecule. It was not surprising that from both substances one gets identic O-methylation products, which prove actually yanangcorinine as isomer of tiliacorinine. The proton correlated NMR at least prove that in yanangcorinine the substituents at C-12 and C-12' have changed (Fig. 14).

Yanangcorinin (**5**)

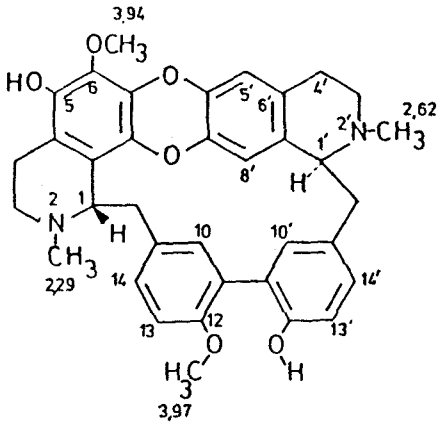


↶ Fernkopplung (2D COSY 45 Experiment)

Fig. 15.

Figure 15 shows the most important long range couplings which are detectable in the 2-dimensional NMR. Because of their short distance the protons 8' and 10' are coupling which- as expected- couple with H-14' and H-13'. On the other side there is a long range coupling of 6-O-methyl group with H-5, so the structure of yanangcorinine is undoubted identified.

Figure 16 shows the second new alkaloid yanangine[13]. The high resolved MS indicates at once that yanangine possesses one oxygen atom more than yanangcorinine. Again the specific rotation hint at the S,S-configuration. Though the proton NMR is quite similar to that of yanangcorinine resp. tiliacorinine there is an important difference: Yanangine shows only signals of eight aromatic protons. The here shown part of the spectrum lacks the signal of the proton at C-5, which indicates at once an additional phenolic OH-group. Actually it was possible to get with diazomethane a mono-O-methylproduct **6a** as well as after longer reaction a O,O-dimethyl-product **6b**. Now we have to discuss the distribution of the both natural O-methyl groups, which should be located at C-5 and C-12' or as here shown at C-6 and C-12. There was quite clear from the MS that one O-methyl group is located at the dibenzo-dioxine part of the molecule and the other at the diphenyl system. The high similarity of the aromatic proton signals in the NMR to the spectrum of tiliacorinine speakes undoubted for a O-methyl group at C-12 like tiliacorinine, so we had to clear only the position of the O-methyl group at the top of the molecule. If these O-methyl groups would be located at C-5 and



Yanangin (**6**)
1S, 1'S
$[\alpha]_D^9$ + 356° ($CHCl_3$)
Molgew: 592 (MS)

^{1}H NMR (δ = ppm)	CH_2N_2		CH_2N_2
	6	**6a**	**6b**
2-NCH_3	2.29	2.29	2.29
2'-NCH_3	2.62	2.64	2.59
5-OCH_3	—	3.82	3.81
6-OCH_3	3.94	3.92	3.91
12-OCH_3	3.97	3.97	3.79
12'-OCH_3	—	—	3.87

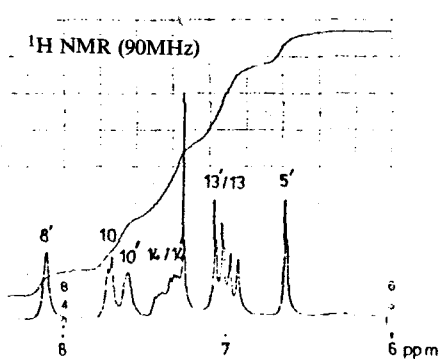
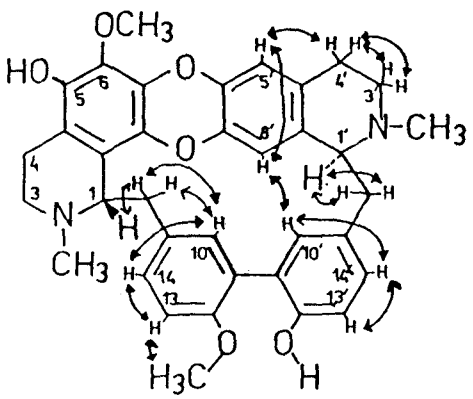


Fig. 16.

therefore the additional OH-group at C-6, then yanangine would be identic with the N,N'-dimethylation-product of pachygonamine, which was recently isolated from *Pachygona ovata*[14]. Indeed all measurements fit relatively well with these of N,N'-dimethyl-pachygonamine. Nevertheless the position of the O-methyl groups in yanangine is C-6

Yanangin (**6**)



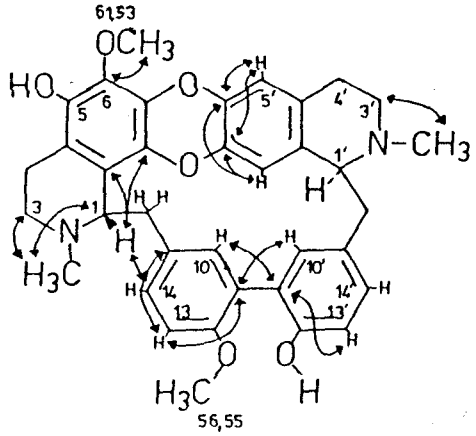
↷ Fernkopplung (2D COSY 45 Exp.)

Fig. 17.

and C-12 as here shown which was proved by the 2-D-NMR spectra.

In the proton shift related NMR spectrum the series of couplings-as indicated here-prove the O-methyl group at C-12, which couple with H-13 followed by a coupling series H-14, H-10, H-α and H-1. On the other side you can see the typical coupling between H-8' and proton 10' and from here to the other protons indicated by a double arrow. The position of the second O-methyl group was proved by the 13-C/1-H -shift correlation NMR (Fig. 17).

Yanangin (**6**)



↷ C-H Fernkopplung (^{13}C-^{1}H Shift Korrellation)

Fig. 18.

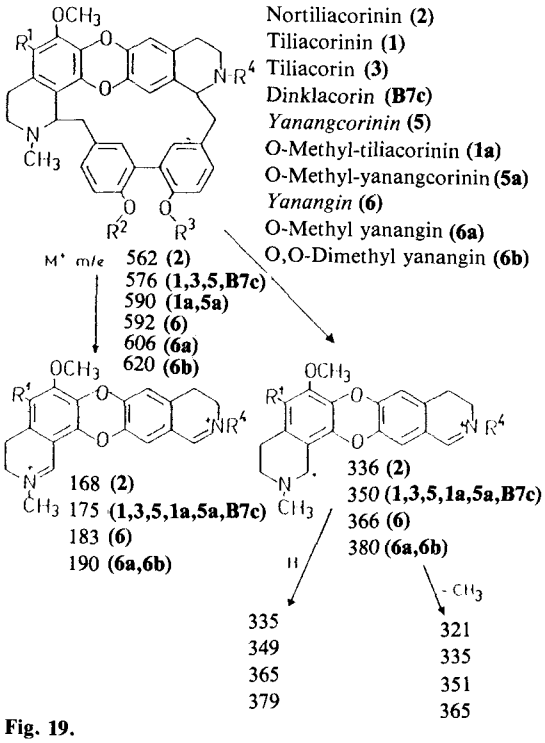


Fig. 19.

An important hint at the O-methyl group at C-6 is one O-methyl signal at 61.53 ppm. Normally the range of aromatic O-methyl signals is near 56 ppm as the signal of the second O-methyl at 56,55. Values near 62 ppm are found if the O-methyl group is in neighbourhood an both sides of an oxygen function-for example as like in tetrandrine. The 13-C/1-H shift correlation NMR spectrum indicates a coupling between the O-methyl group and C-6. So the absolute structure of yanangine was proved without any doubt (Fig. 18).

Figure 19 shows the most important fragments of the MS of these tiliacora alkaloids. Beside the molecule-peak the upper molecule half after loss of the diphenyl part appears as single charged ion as well as the typical double charged ions at the halved m/z values.

From tilianangine we get 22 mg in sufficient purity which allows to clear up the structure[5].

The light blue colour with sulphuric acid containing 1% nitic acid indicates the dibenzo-dioxine structure again, while the specific rotation = +258.6° voted for the S,S configuration found in the main alkaloid tiliacorinine or in yanangine. The proton NMR from tilianangine indicates traces of cyclohexane and dimethylamine which are retained very strong from the macrocyclus. IR and proton NMR prove that there are in tilianangine at least 1 phenolic OH and even 2 N-methyl and 2 O-methyl groups. The here shown range of the 90 MHz NMR is quite similar to that of yanangcorinine; but just so as in yanangine tilianangine missed one aromatic proton signal, which indicates at once an additional OH-group (Fig. 20).

Therefore it was not surprising that the MS from tilianangine and yanangine are identic spectra. The here shown fragmentation pattern proves by high resolution ever 1 OH- and 1 O-methyl- group at the dibenzodioxine part and at the diphenyl system. As usual at the head to head and tail to tail connected biscoclaurine alkaloids the double charged ion appears with strong intensity here at m/z 183 (Fig. 21)

The prove of the structure from tilianangine was got from the proton shift correlation 400 MHz NMR which allowed the here indicated mark of the aromatic protons. Again the typical coupling between H-8′ to H-10′ is followed by an additional coupling series up to the 12′-O-methyl signal which at once prove the 12′-position. A second prove for this 12′-position is the widened doublet of the

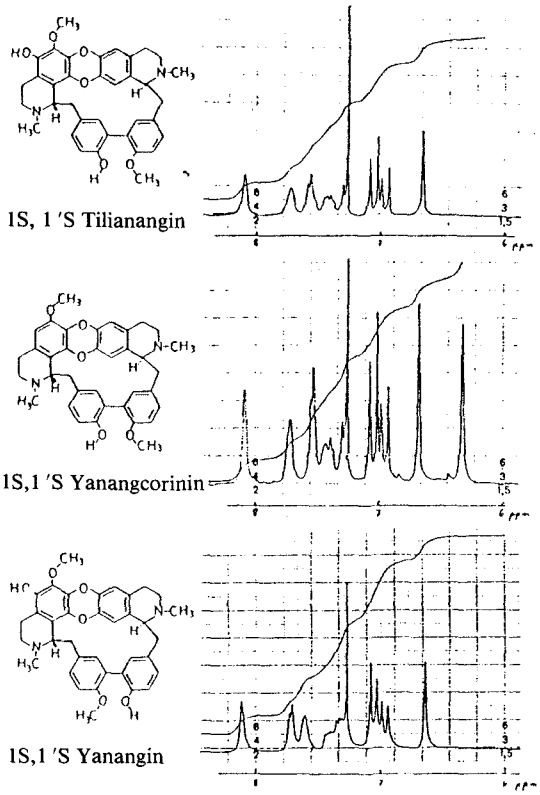


Fig. 20.

MS-Fragmentierung von Tilianangin

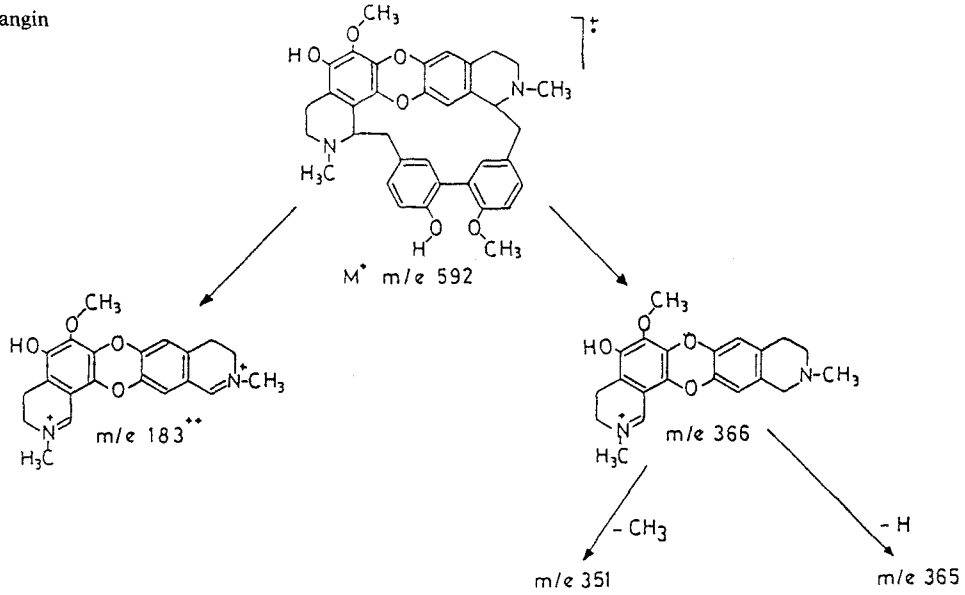
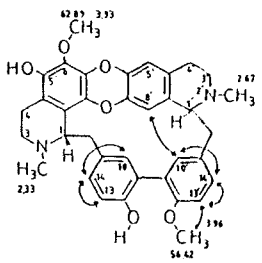


Fig. 21.

H-13′, which is caused by a longe range coupling with the 12-OH group, which is hydrogen-bonded with the 12′-O-methyl group by a twist movement of the diphenyl system. The second O-methyl at C-6 and therefore an OH-group at C-5 can be derived from the 13-C NMR. The ppm-values are quite similar to that of yanangine which is to differ from tilianangine by changing the substituents at position 12 and 12′. As at the isomeric yanangine here a 13-C NMR signal at 15.89 ppm for the C-4 hint at the neighbourhood of the additional OH-group at C-5. The 13-C NMR signal at 62.89 ppm for the 6-O-methyl group hit at the same direction. Therefore with that the structure of tilianangine is absolutely determined (Fig. 22).

Tilianangin



400 MHz

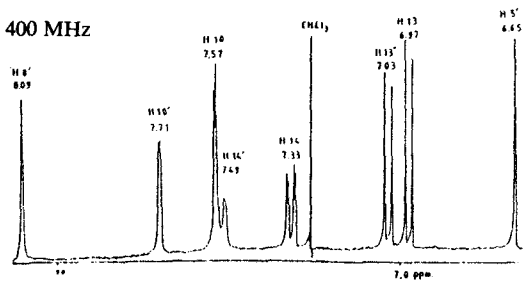


Fig. 22.

The both as well new alkaloids noryanangine and norisoyanangine[16] have nearly the same chromatographic properties as the known tiliageine, which we also found in our alkaloid mixture; therefore it was relatively difficult to isolate these three compounds from each other.

The mass spectra of both new substances were practically identic. High resolution gave the formula and the key fragments *m/z* 352 and *m/z* 176 indicate that two at least for tiliacora new dibenzo -dioxine alkaloids are found which have ever one N-methyl-, O-methyl- and OH-group in the upper half of the molecule. Ever one further OH- and O-methyl-group are positioned at the diphenyl system. Therefore both alkaloids must be norisomers of yanangine (Fig. 23).

The 90 MHz Proton NMR of noranangine in the middle in the range of aromatic protons an

MS-Fragmentierung von Noryanangin u. Norisoyanangin

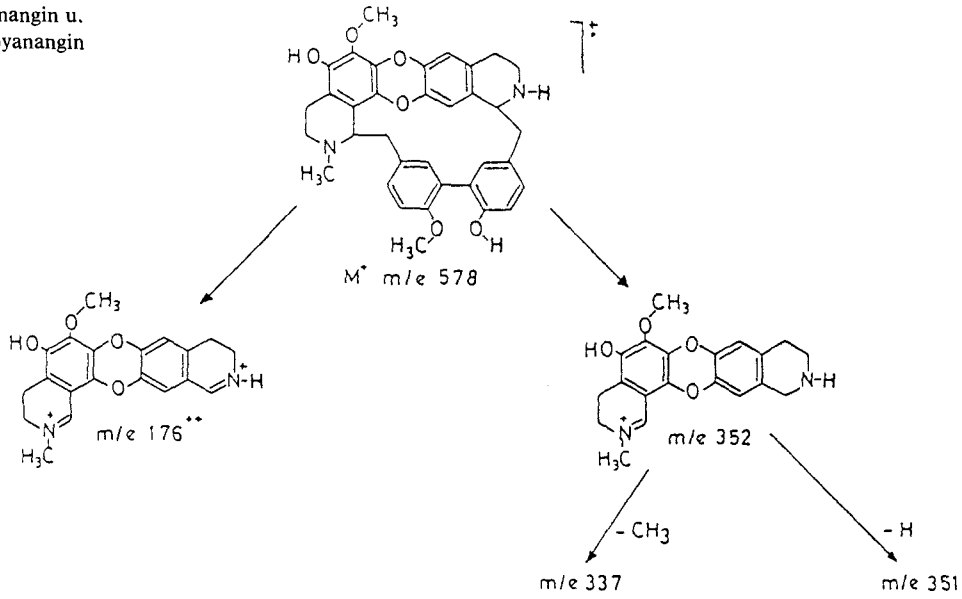
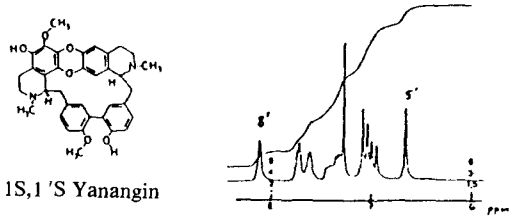
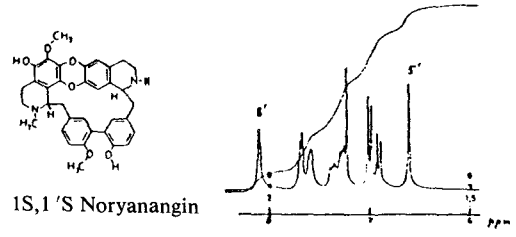


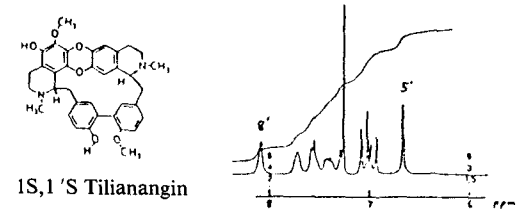
Fig. 23.



1S,1 'S Yanangin



1S,1 'S Noryanangin



1S,1 'S Tilianangin

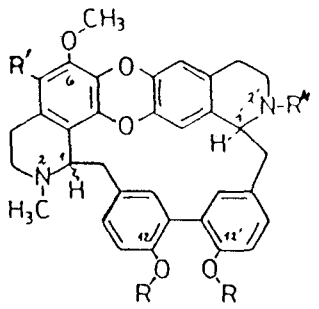
Fig. 24.

stonishingly similarity with yanangine but some clear differences with the NMR spectrum of S,S -tilianangine. The signal of proton 8 ' at 8.11 ppm hit at the S,S or R,R configuration; the first possibility is confirmed by the specific rotation value = +285°. On the other hand noranangine shows a specific rotation of +139°, which indicates at once the R,S-configuration(Fig. 24).

As like as at the left shown alkaloids tiliacorine and nortiliacorine A. This table shows the ppm values of the proton NMR of both alkaloids in relation to other known alkaloids from *Tiliacora triandra*. The good fitting of the values from noryanangine with these of yanangine is obvious. In both new alkaloids the 2 '-N-methyl-signal at 2.67 ppm is absent which prove the 2 '-nor-compound. The additional oxygen function at C-5 caused the relatively deshielding of the 6-O-methyl signal (Fig. 25).

Here is given the mark and split off of the aromatic proton signals from noryanangine which fit very well with yanangine but not with tilianangine (Fig. 26).

The 13-C NMR signals of noryanangine are like these from yanangine, but show in the neighbourhood of the 2 '-N a characteristic shielding effect for C-1 ' and C-3 ' resp. a deshielding effect for C-4 '. This can be seen in the same manner in the 13-C NMR of 2 '-nortiliacorinine as well as at nor-

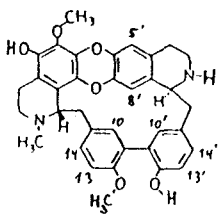


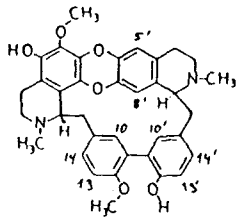
^{1}H NMR-Signallagen (δ = ppm)

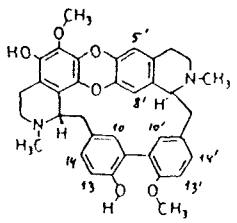
	R,S-Tiliacorin	R,S-Nortiliacorin A	R,S-Norisoyanangin	S,S-Noryanangin	S,S-Yanangin	S,S-Tilianangin
2 NCH_3	2.33	2.31	2.29	2.29	2.29	2.33
2 'NCH_3	2.69	—	—	—	2.67	2.67
6 OCH_3	3.87	3.81	3.92	3.96	3.96	3.93
12 OCH_3	3.97	3.91	3.97	3.99	4.00	—
12 'OCH_3	—	—	—	—	—	3.96
$[\alpha]_D^{20}$	+76.6°	+194.5°	+139°	+285°	+356°	+258.6°
	R' = H	R' = H	R' = OH	R' = OH	R' = OH	R' = OH

Fig. 25.

^{1}H NMR-Signallagen (δ = ppm, J = Hz)



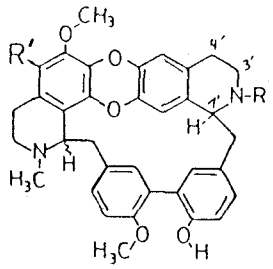




		Noryanangin (200 MHz)	Yanangin (90 MHz)	Tilianangin (400 MHz)
	H 5'	6.64 s	6.64 s	6.65 s
	H 13	6.95 d; J 8.25	6.98 d; J 8.3	6.98 d; J 8.4
	H 13'	6.98 d; J 8.25	7.03 d; J 8.3	7.03 d; J 8.8
⟶	H 14'	7.31 dd; J 8.25/2.1	7.33 dd; J 8.3/1.9	7.52 dd; J 8.8/2.2
	H 14	7.33 dd; J 8.25/2.1	7.36 dd; J 8.3/2.1	7.32 dd; J 8.4/2.2
⟶	H 10'	7.60 d; J 2.1	7.59 d; J 1.9	7.70 d; J 2.2
	H 10	7.69 d; J 2.1	7.69 d; J 2.1	7.53 d; J 2.2
	H 8'	8.11 s	8.11 s	8.10 s

Fig. 26.

isoyanangine. Therefore both new alkaloids are 2'-nor compounds. One O-methyl-signal of noryanangine can be seen at 62 ppm-which indicates an OH-group at C-5 too. Therefore the structure of S,S-2'-noryanangine is proved exactly only from the spectroscopic and MS measurements. For a



^{13}C NMR Signallagen (δ = ppm)

	R,S-Norisoy-anangin	S,S-Noryan-angin	S,S-Nortilia-corinin	S,S-Yanangin	S,S-Tiliaco-rinin
R'	OH	OH	H	OH	H
R	H	H	H	CH_3	CH_3
C1'	58.42	59.91	59.68	67.10	67.36
C3'	45.15	44.26	44.40	52.92	53.08
C4'	29.53	28.59	28.67	26.83	27.19

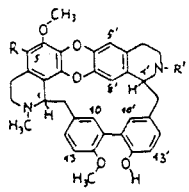
Fig. 27.

N-methylation experiment we did not have enough substance.

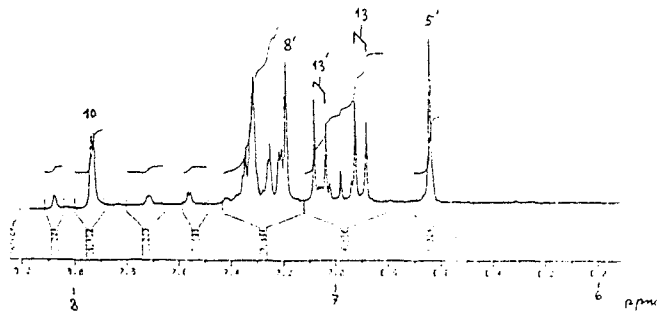
Norisoyanangine shows in the range of the aromatic protons a very strange spectrum, therefore the same substitution pattern like yanangine was difficult to recognize (Fig. 27).

Nevertheless the Figure 28 shows clearly a strong similarity (beside the missed signal of C-5) with the NMR of R,S-tiliacorine, which demonstrate again the R-configuration at C-1. In the group of tiliacora alkaloids with the dibenzo-dioxine structure the proton NMR allows together with the specific rotation to determine the absolute structure of the molecules: R,S-compounds of this type show a characteristic deshielding of the H-10 doublet, while the S,S-alkaloids instead or the H-10 doublet show the singlet of H-8 here. Here too the undoubted mark of the signals was possible by the proton/proton resp. 13-C/proton correlated spectra. This here shown part of the proton NMR of 2' -norisoyanangine prove too a pollution by a second isomeric alkaloid, which we could not separate till now.

The 13-C NMR spectra of these tiliacora alkaloids give a hint at the position of the O-methyl group at C-12 resp. C-12 [12,13,15-17]. The signal of C-12 from yanangine and norisoyanangine is deshielded nearly 1 ppm by the O-methylation, while then the signal C-13 is shielded in relation to the



1R,1'S Norisoyanangin R = OH
R' = H



1R,1'S Tiliacorin R = H
R' = CH_3

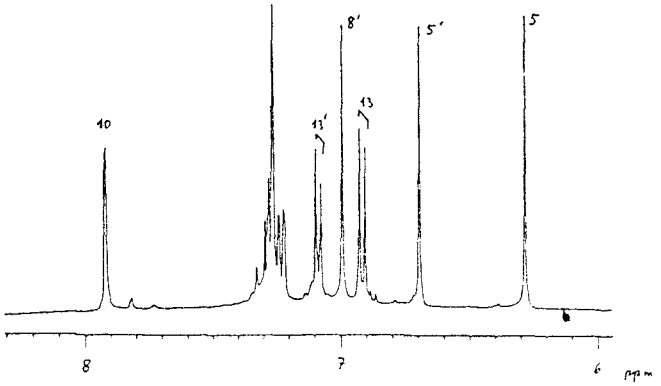
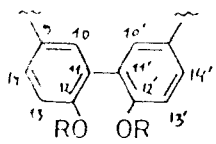


Fig. 28.

^{13}C NMR Signallagen
(δ = ppm)

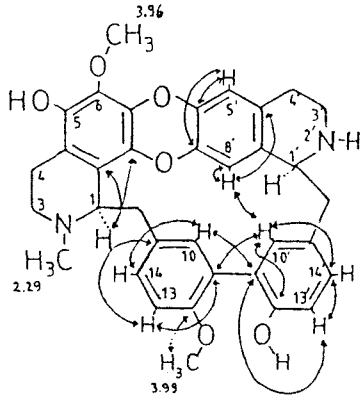


	12 OCH_3:		12'OCH_3:	
	Norisoya-nangin	Yanangin	Tilianangin	Yanang-corinin
C 9	137.32	137.8	136.17	136.37
10	135.38	135.8	135.88	135.88
11	126.32	127.92	127.76	127.79
12	153.21	153.26	152.16	152.1
13	111.19	111.18	112.12	112.03
14	129.8	130.02	130.54	130.31
9'	138.85	136.27	134.3	135.69
10'	134.56	134.46	134.65	134.72
11'	128.03	126.49	126.72	126.75
12'	152.8	152.65	154.1	153.91
13'	118.8	118.53	117.3	117.24
14'	130.4	130.31	130.31	130.02

Fig. 29.

same C-12 and C-13 signals of the here not methylated tilianangine or noryanangcorinine. On the other hand the same situation is recognizable at C-12' and C-13'. These signals are not exchangeable but confirmed by 13-C/proton correlation spectra (Fig. 29).

Figure 30 shows the important long range couplings which we found by the 13-C/H shift correlation spectra and proton/proton shift correlation as well. The characteristic interaction between H-8' and H-10' of these dibenzodioxine alkaloids here



2' Norisoyanangin

Fig. 30.

also allows to fix the mark of the aromatic protons and from here the mark of the corresponding 13-C signals without any further chemical derivation, so the structure of 2'-norisoyanangine is established.

The next new alkaloid tilitriandrine I like to speak about is relatively similar to the known alkaloid tiliageine. Both alkaloids don't reach with sulphuric acid containing 1% nitric acid, therefore it was quite clear that these compounds have no dibenzo-dioxine system.

Tiliageine is known from tiliacora dinklagei[18] which we have also found in *Tiliacora triandra*. As expected the nor-compound tilitriandrine give a MS which is except the lacking N-methyl group quite similar to the MS of tiliageine. It seems to be possible that tilitriandrine might be a 2'-nor-tiliageine which is unknown til now (Fig. 31).

Figure 32 shows a table of physical data of tilitriandrine in relation to these of tiliageine and the recently published antioquine, which hint at the correct structure of tilitriandrine as 2'-nor-antioquine. Again these two known alkaloids tiliageine and antioquine differ only with the substituents at C-12 and C-12'.

We have had enough tilitriandrine to get 13-C/1-H and proton/proton shift correlation NMR spectra. The 13-CNMR shows three O-methyl signals nearly at 56 ppm which prove that both O-methyl groups at the top of the molecule must be in position 6 and 6'. From the shift correlation

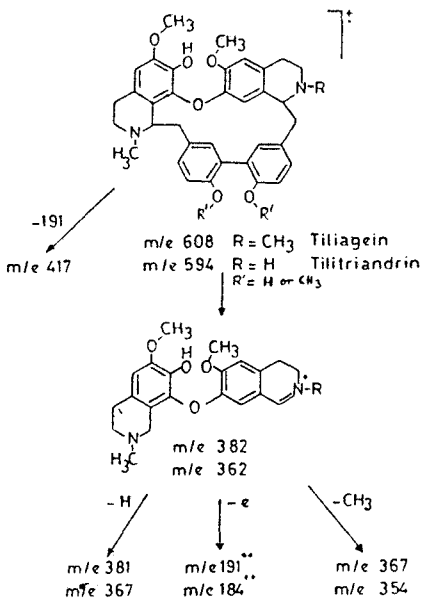
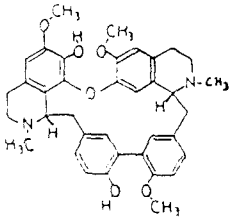
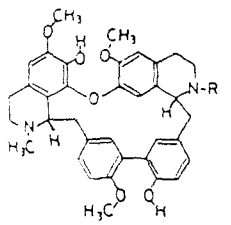


Fig. 31.



1S,1'R Tiliagein

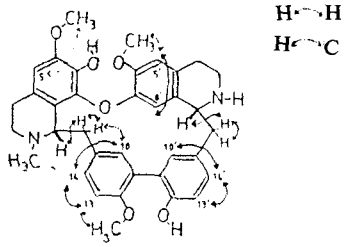


1S,1'R Tilitriandrin R = H
Antioquin R = CH_3

	Tiliagein	(lit.)	Tilitriandrin	Antioquin	
2-NCH_3	2.34	2.34	2.36	2.34	
2'-NCH_3	2.62	2.60	—	2.63	
6-OCH_3	3.45	3.41	3.48	3.49	
6'-OCH_3	3.79	3.76	3.84	3.84	
12-OCH_3	—	—	3.88	3.87	
12'-OCH_3	3.84	3.81	—	—	
[α]	179.1°×	132.6°××	214°×	198°×	×$CHCl_3$
Schmp	270°	270-272°	192°	197°	××Pyridin

Fig. 32.

spectra the here shown interactions respectively couplings are to recognize. Because the more instability of the macrocyclus here the useful interaction between H-8' and H-10' is not detectable. The mark of all signals was possible starting the couplings series with the signals of H-1 on the left side. It



B2 = Tilitriandrin

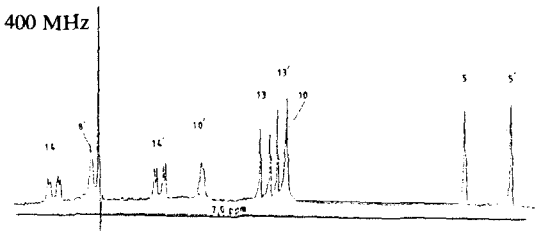
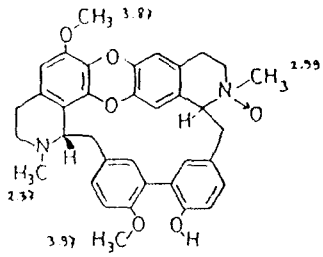
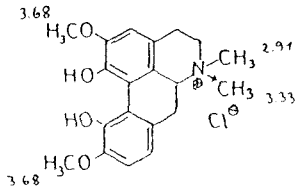


Fig. 33.



1S 1S Tiliacorinin 2'N oxid



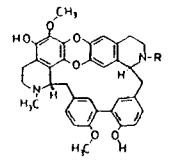
Magnoflorin

Fig. 34.

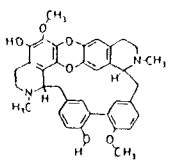
has to be explained the difference of the intensity respectively the half width value of the H-13 signals. The doublet of H-13 is weaker and broadened than the doublet of H-13'. Analogously to the situation at yanangcorinine and tiliacorinine this difference give a hint at the O-methyl group at C-12, therefore the new tilitriandrine is really a 2'- norantioquine (Fig. 33).

The last two alkaloids which we isolated from *Tiliacora triandra* were the known 2'-N-oxide of tiliacorinine and magnoflorine[6] which is a characteristic alkaloid of all Menispermaceae. Tiliacorinine-2'-N-oxide was detected by the characteristic M-16 signal of the MS and as well with the deshielded 2'-N-methyl NMR signal. By reduction with sulphurous acid we got as expected tiliacorinine. Magnoflorine was isolated as Reinecke salt and in relation to authentic material identified.

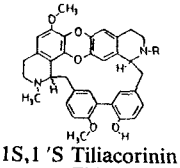
Figure 35 shows again all bisbenzyl-isoquinoline alkaloids which we isolated from *Tiliacora triandra*, 6 of these are new compounds. Except of the both alkaloids tiliageine and tilitriandrine all compounds possess the dibenzo-dioxine partial structure. As like tetrandrine this type of tiliacora alkaloids should have bactericidal and cytostatic activities. It is known that the O-methyl derivative of tiliageine named funiferine is active against lympholytic leucaemia P 388. The pharmacological screening of tiliacorinine and yanangcorinine as well seems to be very interesting, because these alkaloids are quite similar to S,S-trilobine which



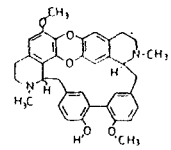
1S,1 'S Yanangin R = CH_3
Noryanangin R = H
1R,1 'S Norisoyanangin R = H



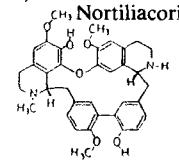
1S,1 'S Tilianangin



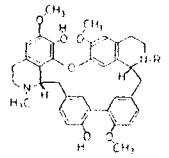
1S,1 'S Tiliacorinin R = $-CH_3$
Tiliacorinin-N-Oxid R = $-CH_3$
Nortiliacorinin A R = H
1R,1 'S Tiliacorin R = $-CH_3$
Nortiliacorin A R = H



1S,1 'S Yanangcorinin
1R,1 'S Dinklacorin



1S,1 'R Tilitriandrin



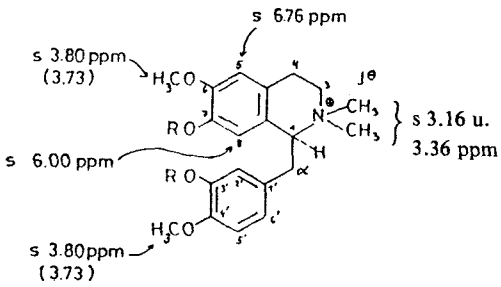
1S,1 'R Tiliagein
R = $-CH_3$

Fig. 35.

have *in vivo* a strong antitumor activity as tetrandrine. A very important hindrance is the difficulty to get enough material of all interesting side alkaloids.

The third Menispermaceae genus which we investigated was tinospora. From *Tinospora cordifolia* (in Thailand known as chinchachalee) which was as incorrect labeled as Krung Kha Mao and which is really *tinospora baenzigeri* Forman[19], we have isolated two quaternary alkaloids over the Reinecke salts[20].

The first was the known benzyltetrahydro-isos-



Tembetarin: R = H $FeCl_3$, Gibbs R, ++; s 8.99 ppm
UV λ_{max}: 210, 228, 284 nm
N-Methyl-Laudanosin: R = CH_3 s 3.30 u. 3.68 ppm

Fig. 36.

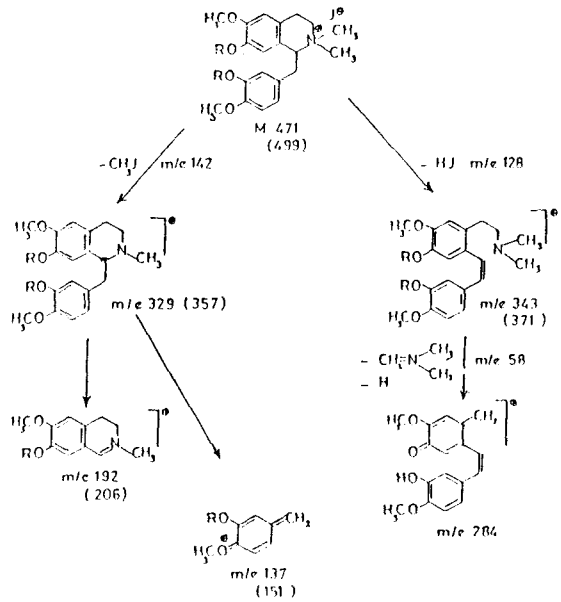
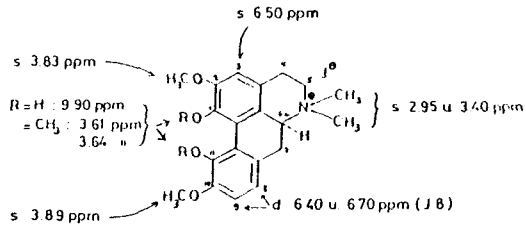


Fig. 37.

quinoline derivative tembetarine which was identified in addition to the physical data as shown here by the O-methylation into the well known N-methyl -laudanosine (Fig. 36).

Figure 37 shows the MS fragmentation pattern of tembetarine. Characteristical for these quaternary alkaloids is not only the loss of methyliodide or hydroiodide but also the fragmentation of m/z 58 resulting the ion m/z 284. The second alkaloid here was again magnoflorine which is shown in figure 37 with the physical data which we got by the different measurements. The MS fragmentation pattern of magnoflorine is shown in figure 39 with the characteristic key fragments as we have seen at tembetarine.

The last example of Menispermaceae drug I like to tell about is Tinospora crispa, a traditional drug of Indonesia which is used there mainly against



Magnoflorin R = H $FeCl_3$, Gibbs R. ++
UV λ_{max}: 224, 271, 307 nm

Fig. 38.

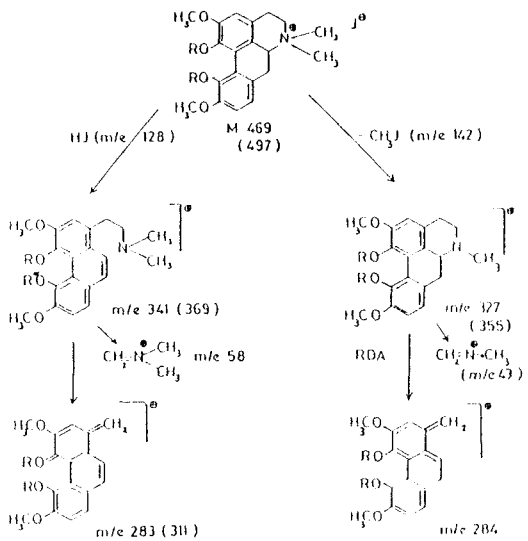


Fig. 39.

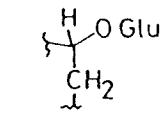
gastro intestinal deseases. In relatively high contents we have found a bitter tasting glycoside which we named tinocrisposide[21] (Fig. 40).

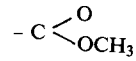
Tinocrisposide has the formula C_{27} H_{36} O_{11} which we got by elementary analysis and by the MS spectra. With acetic anhydride we got a tetracetate.

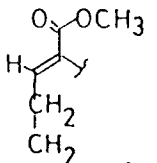
Tinocrisposid
$C_{27}H_{36}O_{11}$ (536.58) (FAB)
Aglyson: $C_{21}H_{25}O_6$ (EIMS)
Schmp.: 119-120° (Methanol)
$[\alpha]_D^{21}$: -23.8° (c 1.0; Ethanol)
DerivaL: TetraacetaL

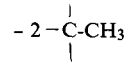
Partialstrukturen:

- Furyl
- Lacton
- Glucose
- 4 CH_2-Gruppen









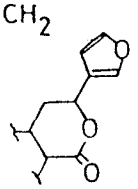
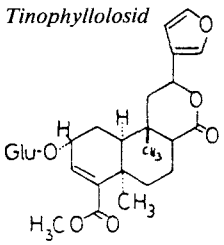


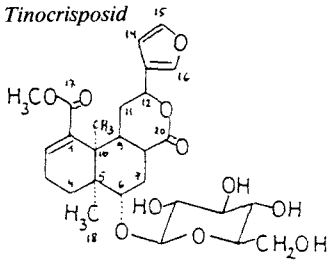
Fig. 40.

Tinophyllolosid



$C_{27}H_{36}O_{11}$
Schmp.: 155-158° (155-138°)
$[\alpha]_D^{21}$: -19.2°
H. Itakawa *et al.*
Phytochemistry 25, 905 (1986)

Tinocrisposid



$C_{27}H_{36}O_{11}$
Schmp.: 119-120°
$[\alpha]_D^{21}$: -23.8° (c = 1.0; Ethanol)

Fig. 41.

Cyclea barbata Miers
K. Dahmen
C. Goepel
B. Hoffstadt
S.v. Kuerten
H.J. Martin
D. Moecke
M. Praest
T. Yupraphat
P. Zymalkowski
Tiliacora triandra Diels
T.J. Tan
H. Khosravian
Tinospora (cordifolia) baenzigeri Forman
C. Schneider
Tinospora crispa (L.) Hook f. + Thoms
A.Z. Adnan

Fig. 42.

From the spectra we identified these partial structures as shown in Fig. 40.

The result of all is the proposed structure which is on the right side of figure 41.

Though tinoscrisposide has the same functional groups as the recently published tinophylloside which was isolated from another genus of Menispermaceae there are clear differences between both natural products.

At last I have to thank my coworkers which take the decisive part in these investigations and which are listed in figure 42.

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