

CYCLOPEPTIDE ALKALOIDS CONTAINING A STYRYLAMINE RING UNIT <sup>+)</sup> Rudolf Tschesche*Institut für Organische chemie und Biochemie der Universität Bonn, BRD*

The area of cyclopeptide alkaloids<sup>1</sup> containing a styrylamine unit in the ring has developed very quickly in the last ten years, and over 70 compounds of this type are known. Three groups having 13-, 14- and 15-membered ring systems are to distinguish. Alkaloids with a 14-membered ring system are the main type. The first representatives having such a ring were isolated in 1963 by Goutarel and Pais in Gif-sur-Yvette, France, from the plant *Waltheria americana*. They reported later the correct formulas of the three compounds designated adouétine-X, -Y and -Z. In 1966 they could isolate pandamine and pandaminine from the plant *Panda oleosa* and proposed the complete structures. Derivatives of the 13-membered ring type were found in *Ziziphus oenoplia* and named ziziphine and ziziphinine by Zbiral et al., though the supposed structures have later to be revised. Correct formulas for compounds of the 13- and 15-membered alkaloids were first determined by Tschesche et al. in 1973-1974. The major contributions on these alkaloids have been made by the groups working in Paris and Bonn, while others have done some important works in this field.

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 +) This paper is dedicated with the best wishes Prof.T.Takemoto on the occasion of his retirement.

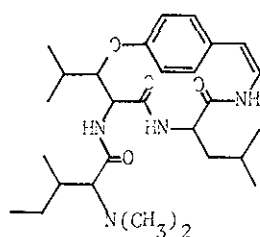
The type of cyclopeptide alkaloids are found particularly in plants of the family Rhamnaceae and occasionally in such of Celastraceae, Hymenocarpaceae, Pandaceae, Rubiaceae, Sterculiaceae and Urticaceae, however, the distribution in the plant kingdom was not carefully investigated. The compounds are present in the leaves and in the barks and occur in the most cases as a complex mixture of more than five constituents difficult to separate. The total yields from dried plant materials may differ between 0.01 to 0.1%. The ratios of various alkaloids and their distribution in the plant may differ extremely with the region of the growth, the season, the maturity of the plant and other unknown factors. This is a serious drawback for investigations.

The isolation of pure alkaloids was achieved from ground plant material by extraction with a mixture of benzene, concentrated aqueous ammonia and methanol in the ratio of 100:1:1. From the combined benzene extracts the rough bases are obtained by shaking with 5% aqueous citric acid. The isolation of the pure alkaloids is achieved predominately by repeated chromatography on silica gel columns or plates with different solvent systems. For examination and identification of the isolated compounds mass spectrometry is the method of choice combined with other optical techniques.

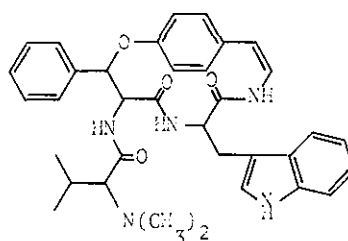
These alkaloids are rather weak bases and the solubility in the water is poor. Most are isolated in crystalline form but some can be obtained only in amorphous state. The optical rotation is generally levorotatory ( $-200 \sim -400^\circ$ ).

The individual cyclopeptides differ in the nature of their amino acid constituents, however, amino dicarboxylic acids, diamino acids and sulfur containing amino acids are not found. Further differences occur in the ratio of methylation (mono- or dimethylated) and in the position of the keto group in the molecules. In the 13- and 15-membered types a methoxy- or hydroxy substitution in the aromatic nucleus of the styrylamine part is observed.

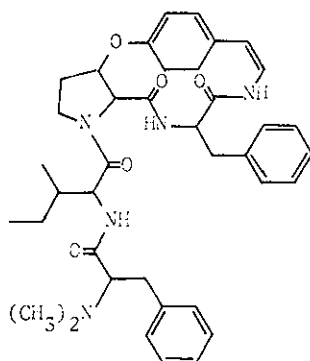
Up to date the alkaloids containing a 14-membered ring are the largest group with more than 45 representatives known.<sup>2b</sup> It can be subdivided into three subgroups depending on the  $\beta$ -hydroxy amino acid participating on the ring formation, namely  $\beta$ -hydroxyleucine (frangulanine-type (1)),  $\beta$ -hydroxy-phenylalanine (integerrine-type (2)) and trans-3-hydroxyproline (amphibine-B-type (3)). Bases with  $\beta$ -hydroxyvaline (hymenocardine (7)) and with  $\beta$ -hydroxy-isoleucine (ceanothine-D) as ring constituents are rare.



(1) frangulanine



(2) integerrine



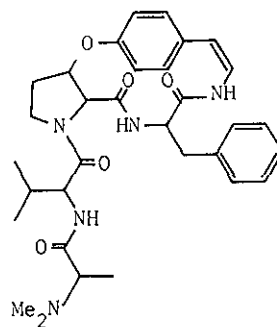
(3) amphibine-B

#### 14-membered ring type

The formulas demonstrate that in the ring formation besides the hydroxystyrylamine unit and a hydroxyamino acid a further different amino acid is participating (ring bond amino acid). The carboxyl group of the hydroxyamino acid is linked with a second amino acid which is mono- or dimethylated (side chain amino acid). In some cases the carboxy group of the side chain amino acid is forming a peptide bond with a further amino acid, which is methylated (see amphibine-B). The "intermediate" amino acid is inserted between the hydroxyamino acid and the basic end amino acid within the peptide chain.

The representatives of this group generally show no characteristic uv absorption in spite of the double bond and the conjugated aromatic styrylamine part. The reason for this is explained by the strained character of the ring which prevents overlap of the p-orbitals of the enamine and the styryl group. Thus both absorptions were found to be independent. This assumption was confirmed by a X-ray structure analysis of mauritine-A ((4) and fig.1)<sup>2c</sup>. In addition this fact explains also that in the pmr-spectrum the absorption of the 3-hydroxyproline is in conformity with the cis form but in reality it exists in the trans form and indeed the trans-3-hydroxyproline was isolated after precautious degradation.

The amino acids in all three groups of cyclopeptide alkaloids have predominately the L-configuration, however, in lasiodine-A (8), isolated by Goutarels group, the amino acid in the ring is D-phenylserine and in scutianine-D and -E D-threo and D-erythro- $\beta$ -hydroxyleucine were observed by us in the same posi-



(4) mauritine-A

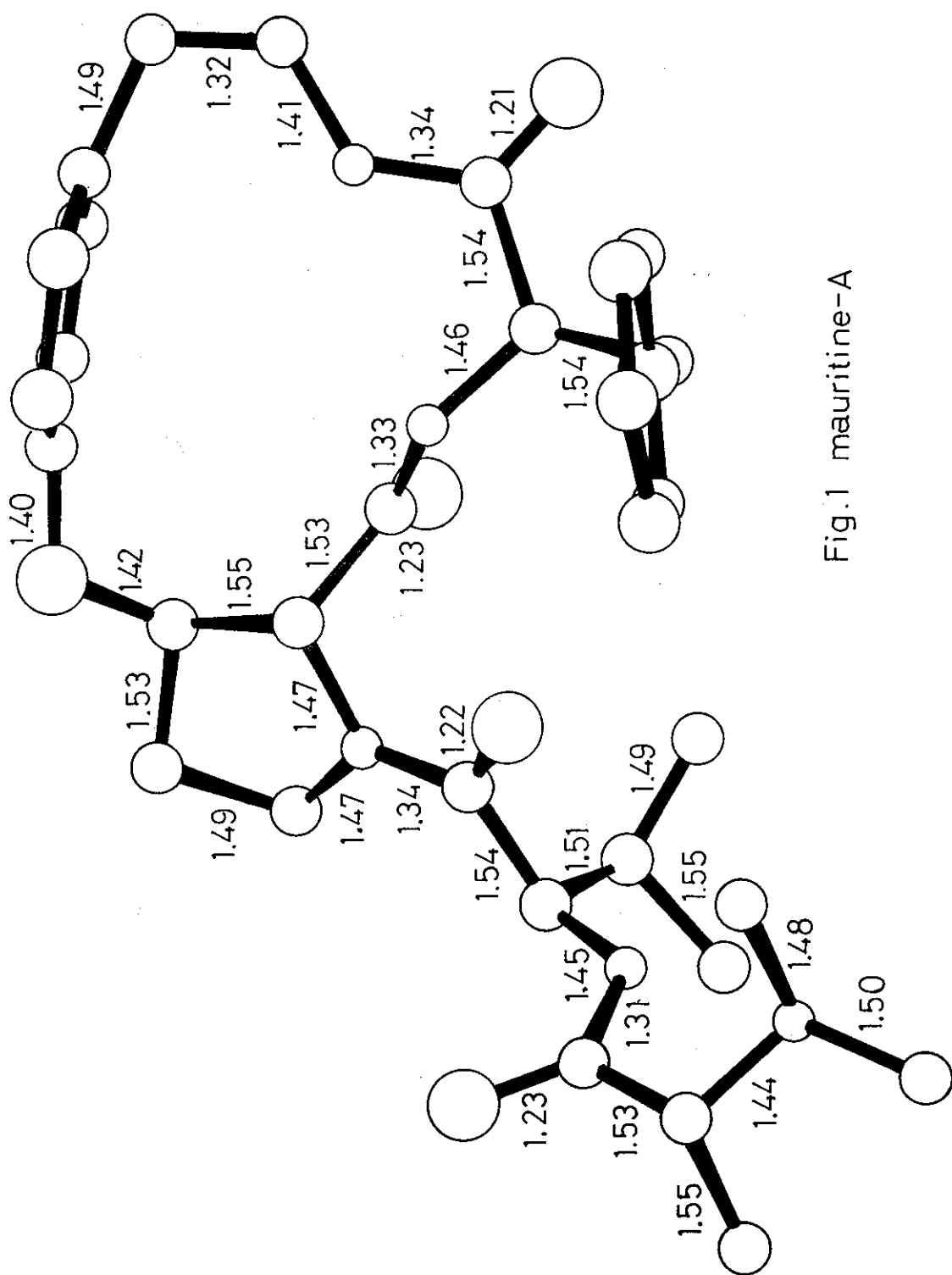


Fig.1 mauritine-A

tion. The attribution to the L- or D-series was found on reactions with specific amino acids oxidases.

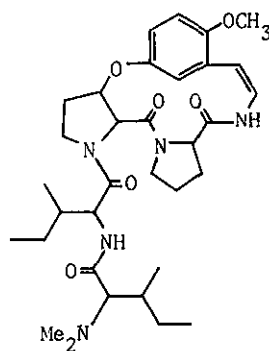
Another problem in the frangulane type alkaloids was the configuration of the hydroxyleucine. Acid hydrolysis of these compounds produced the threo-form, but correct is the erythro-isomer. This was established with different methods by several authors. It seems likely that the hydroxyphenylserine in the integerrine group has the erythro form as well.

In the amphibine-B group the characteristic ring bond hydroxyamino acid is trans-3-hydroxyproline. This amino acid rarely occurs in the animal kingdom as constituent of proteins and has been found in plants only once in its free form in *Delonix regia* Rafin. The isolation of trans-3-hydroxyproline was possible after ozonolysis of the aromatic nucleus followed by hydrolysis under mild conditions.

#### 13-membered ring type

Alkaloids of this group contain  $\beta$ -(2-methoxy-5-hydroxy)-styrylamine instead of the 4-hydroxystyrylamine. A typical compound of this type is ziziphine-A (5). Eight representatives are known to date<sup>1,2a,2b</sup>.

Oxidative degradation of ziziphine-A with osmium tetroxide and sodium periodate yields a dialdehyde, since one aldehyde group is formed at the amino group and becomes easily hydrolyzed, thus producing the corresponding amino monoaldehyde. The uv spectrum is very similar to 2,5-dimethoxybenzaldehyde. Acid hydrolysis of the dihydroziziphine-A produced a di-



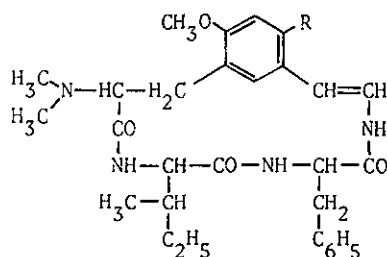
(5) ziziphine-A

peptide derived from the side chain and its hydrolysis yielded isoleucine. Therefore isoleucine is situated on this part of the molecule. Alkaline hydrolysis of the hydrogenated compounds of this type produced 8-(2-methoxy-5-hydroxyphenyl)ethanol, which indicates the position of the methoxy substitution in the styrylamine part in these alkaloids. Trans-3-hydroxyproline is the hydroxyamino acid in all the bases of this type.

#### 15-membered ring type

Twelve representatives of this group have been isolated from different ziziphus varieties. The ring is here closed by direct substitution of the aromatic nucleus of the styrylamine by an amino acid instead of the usual ether linkage. It seems likely that from an unknown precursor the hydroxyl group of a serine derivative has been eliminated with a hydrogen of the aromatic ring in form of water. A typical compound of this type is mucronine-A (6).

From this group the first time non-methylated amino acids adjacent to the aromatic nucleus of the styrylamine part have been isolated. These compounds are not stable enough for a detailed structural investigation, but can be stabilized in form of the acetyl derivatives. In the representatives of the 15-membered group a new



(6) mucronine-A (R=H)

unknown bisamino acid is present, though it was not possible to isolate this compound after acid hydrolysis. Oxidative cleavage with osmium tetroxide and sodium periodate produced an aminodialdehyde which were analogs of the oxidation products of the 13-membered alkaloids. Lost of the C-atom at the amino group

yielded an amino-mono-aldehyde. Hydrolysis generated a dipeptide but not the unknown aromatic bisamino acid. The mode of substitution in the aromatic nucleus by methoxy group can be revealed by comparison of the uv- and pmr-spectra with those of the corresponding dimethoxy- and methoxymethylbenzaldehydes.

In the case that monomethylated alkaloids are present their identification can be relieved by reaction with formaldehyde followed by catalytic hydrogenation. This procedure is applicable to all alkaloids in this area.

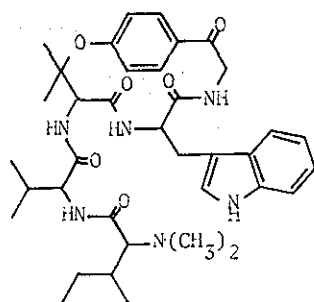
#### Lasiodyne-A, an open chain alkaloid

The only non-cyclic peptide alkaloid known is lasiodine-A (8), having most of the structural features of the whole group. Warnhoff<sup>1</sup> has postulated that it might be formed from a cyclic precursor like hymenocardine (7), which was isolated by Goutarel et al. from *Hymenocardia acida*, while lasiodine-A was produced in *Lasiodyneus marmoratus*. Hymenocardine is the only peptide derivative with a  $\beta$ -hydroxyvaline as ring forming hydroxyamino acid. For the origin of lasiodine-A is indicated the mild alkaline hydrolysis which opens the ring on the position of  $\beta$ -hydroxyvaline forming a free phenolic hydroxy group. The hydroxyvaline part of the molecule easily forms an unsaturated compound by loss of water in the same reaction as in lasiodine-A occurs.

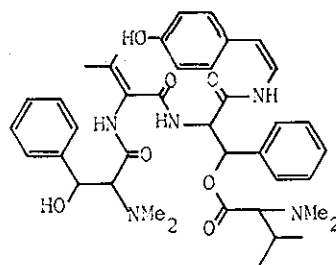
Most structures of the cyclopeptide alkaloids have been determined by mass spectrometry using high resolution<sup>1</sup>. The three major groups differ in the pattern of fragmentation and in the distinct stability of the ring against electron impact. In combination with chemical methods a correct determination of the structure is possible even if only small amounts of starting material are available.

A micro method for the determination of the structures in the styrylamine



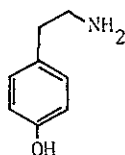


(7) hymenocardine

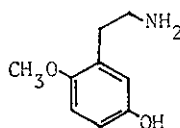


(8) lasiodine-A

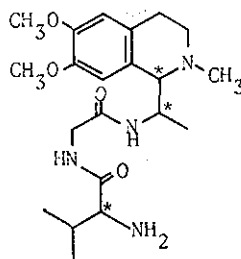
residue of the 13- and 14-membered cyclopeptide alkaloids has been developed by means of gas chromatography. The hydrogenated bases are hydrolyzed to the splitting products (9) and (10).



(9)



(10)



(11) amphibine-I

The latter is predominately produced from the 13-membered type. Both hydrolytic products can be separated as trimethylsilyl derivatives in the gas chromatography. Comparison of the retention times of authentic samples with those of the derivatives from the alkaloids allows to determine the substitution pattern of the aromatic nucleus in the styrylamine residue<sup>2d</sup>.

Little is known about the biogenesis of the cyclopeptide alkaloids. Interestingly is that in some cases tetrahydroisoquinoline alkaloids have been found in the plants together with the cyclopeptide derivatives such as R-(-)-arnepavine, nuciferine and  $\beta$ -methylcocclaurine. In *Ziziphus amphibia* the

the peptide of a tetrahydroisoquinoline, amphibine-I (11), was observed<sup>3</sup>. The structure was confirmed by synthesis<sup>4</sup>. Bishay et al.<sup>1</sup> pointed out these compounds could correlate with the biogenesis of the cyclopeptide alkaloids. This hypothesis has still to be investigated.

#### References

- 1 More detailed abstract on cyclopeptide alkaloids: R.Tschesche and E.U.Kaussmann "The Alkaloids" by R.H.F.Manske, Academic Press, New York, 1975, Vol.XV
- 2 Newer publications in press:
  - a) Nummularine-D, -E and -F, new cyclopeptide alkaloids from *Ziziphus nummularia*: cf. R.Tschesche, M.Elgamal, G.A.Miana and G.Eckhardt, Tetrahedron
  - b) Jubanin-A and -B, new cyclopeptide alkaloids from *Ziziphus jujuba* Mill.: cf. R.Tschesche, I.Khokhar, H.Wilhelm and G.Eckhardt, Phytochemistry
  - c) A.Krifel, G.Will, R.Tschesche and H.Wilhelm, Die Kristallstruktur des Mauritins-A: cf. Phytochemistry
  - d) U.Henke, Diplomarbeit Univ. Bonn 1975
- 3 R.Tschesche, C.Spilles and G.Eckhardt, Chem.Ber., 197, 1329 (1974)
- 4 R.Tschesche, J.Moch and C.Spilles, Chem.Ber., 198, 2247 (1975)

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