Summary

Chromatographic resolution of the bark of Z.rugosa(Rhamnaceae) on SiO₂ gel resulted in the isolation of five cyclopeptide alkaloids and three flavonoids provisionally designated as Zr-1,Zr-2,Zr-3,Zr-4,Zr-5,Zr-6,Zr-7 and Zr-8 by a procedure adopted by R.Tschesche etal.

Zr-1

The alkaloid Zr-1 is colourless granules,mp 264-265⁰C[α]₀²⁵-210(CHCl₃,C 0.5) was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum.IR spectrum exhibited bands for –NH, -CH , -NMe,- NHCO,Ar-O-C and double bond. On acid hydrolysis it give phenylalanine and N,N-dimethylisoleucine. The mass spectrum of Zr-1 showed a close similarity to that of Scutianine-C ,a known cyclopeptide alkaloid.

Examination of spectral data and hydrolyses experiment shows that Zr-1 possesses structure(1). A scrutiny of IR,UV,HRMS and hydrolysis experiment revealed that the structure(1) proposed for Zr-1 is identical to that of Scutianine-C,a known cyclopeptide alkaloid reported from Scutia buxifolia.

Finally Zr-1 was proved to be identical with Scutianine-C by direct comparison with authentic sample(Co-TLC,mmp)
Zr-2

The alkaloid Zr-2 is colourless granules, mp 103-104°C [α]D^25 - 315° (c 0.33 MeOH) was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum. The IR spectrum exhibited bands for -NH, -CH, -NMe, -NHCO, Ar-O-C and styryl double bond. On acid hydrolysis, it gave alanine and N,N-dimethylalanine. The mass spectrum and ^1H NMR spectrum showed a close similarity to that of Mauritine-A, a known cyclopeptide alkaloid reported from Z. mauritiana. Examination of spectral data and hydrolysis experiments shows that Zr-2 possesses structure (1). A scrutiny of IR, UV, HRMS, ^1H NMR and hydrolysis experiments revealed that the structure 1 proposed for Zr-2 is identical with that of Mauritine A, a known cyclopeptide alkaloid reported from Z. mauritiana.

Finally Zr-2 was proved to be identical with mauritine-A by direct comparison with authentic sample (co-TLC mmp and IR).

Zr-3

The alkaloid Zr-3 is amorphous powder [α]D^25 -256° (c 0.1 CHCl₃) was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum. The IR spectrum exhibited bands for -NH, -CH, -NMe, -NHCO, Ar-O-C and styryl double bond. On acid hydrolysis it gave isoleucine, leucine and N,N-dimethylisoleucine. The mass spectrum and ^1H NMR spectrum showed a close
similarity to that of Mauritine-D, a known cyclopeptide reported from Z. mauritiana. Examination of spectral data and hydrolysis experiments show that Zr-3 possesses structure (1). A scrutiny of IR, UV, HRMS, $^1$H NMR and hydrolysis experiments revealed that the structure (1) proposed for Zr-3 is identical with that of Mauritine-D, a known cyclopeptide reported from Z. mauritania.

Finally Zr-3 was proved to be identical with mauritine-D by direct comparison with authentic sample (co-TLC, mmp and IR).

**Zr-4**

The alkaloid Zr-4 is amorphous powder $[\alpha]^{25}_D$-203$^0$ (c 0.05 MeOH) was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum. The IR spectrum exhibited bands for $-\text{NH}$, $-\text{CH}$, $-\text{NMe}$, $-\text{NHCO}$, Ar-0-C and styryl double bond. On acid hydrolysis it gave phenylalanine, N,N-dimethylphenylalanine and isoleucine. The mass spectrum and $^1$H NMR spectrum showed a close similarity to that of Amphibine-B, a known cyclopeptide alkaloid reported from Z. amphibia. Examination of spectral data and hydrolysis experiments show that Zr-4 possesses structure (1). A scrutiny of IR, UV, HRMS, $^1$H NMR and hydrolysis experiments revealed that the structure (1) proposed for Zr-4 is identical with that of Amphibine-B, a known cyclopeptide alkaloid reported from Z. amphibia.
Finally Zr-4 was proved to be identical with amphibine-B by direct comparison with authentic sample (co-TLC, mmp).

Zr-5

The alkaloid Zr-5 is colourless granules $\left[\alpha\right]^{25}_{D}-43^0 (c 0.06\text{ MeOH})$ was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum. The IR spectrum exhibited bands for $-\text{NH},-\text{CH},-\text{NMe},-\text{NHCO Ar-O-C}$ and styryl double bond. On acid hydrolysis it gave 3-hydroxy leucine and leucine. Alkaloid Zr-5 on further hydrolysis with $\text{Ba(OH)}_2$ furnished $\text{N,N}$ dimethyltryptophan. The mass spectrum and $^1\text{H NMR}$ showed a close similarity to that of Nummularine-K, a known cyclopeptide alkaloid reported from $Z$-nummularia. Examination of spectral data and hydrolysis experiments show that Zr-5 possesses structure (1). A scrutiny of IR, UV, HRMS, $^1\text{H NMR}$ and hydrolysis experiments revealed that structure (1) proposed for Zr-5 is identical to that of nummularine-K, a known cyclopeptide alkaloid reported from $Z$-nummularia.

Finally Zr-5 was proved to be identical with nummularine-K by direct comparison with authentic sample (co-TLC, mmp and IR).
Zr-6

Compound Zr-6 as yellow granules, m.p. 226-228°C, C_{16}H_{12}O_{6} (M^{+}, 300.0632) was found to be a flavone on the basis of positive Shinoda test and UV spectrum. It showed bands for phenolic -OH and chelated carbonyl groups in the IR spectrum.

**Methylation of Zr-6**

Zr-6 on methylation with CH_{2}N_{2} in chilled solvent ether gave two methyl derivatives, trimethyl derivative, m.p. 148-150°C (^{1}H NMR, δ 3.88, 4.15, 4.24 for 3X-OMe) and tetramethyl derivative, m.p. 145-146°C (^{1}H NMR, δ 3.89, 4.16, 4.28, 4.32 for 4X-OMe). This clearly indicated that Zr-6 contains either four hydroxyl groups or three hydroxyl groups and one methoxyl group. As the molecular formula of Zr-6 is C_{6}H_{12}O_{6} which contain one more carbon than the flavonoid nucleus indicated the presence of one methoxyl and three hydroxyl groups in the molecule. The presence of one -OMe group in Zr-6 is also supported by a peak at δ 3.85 in the ^{1}H NMR spectrum. In order to locate the position of 3-OH and OCH_{3} groups, the UV spectrum of Zr-6 with different shift reagents were studied in detail. An analysis of all the UV spectrum revealed the following facts for the structure of Zr-6

i) Zr-6 is a flavone or flavonol

ii) Absence of free-OH at C-4
iii) It is a 3,5-dihydroxy flavone

iv) There is free –H group at C-7

v) No orthodihydroxy (catechol) system present.

vi) The –OMe group at C-4 position.

Thus, the structure of Zr-6 was characterised as Kaempferol-4’-methyl ether by chemical and spectral data and direct comparison (co-TLC and mmp) with an authentic sample. This is the first report of the compound in Z. rugosa.

Zr-7

Compound Zr-7 as yellow granules, m.p. 320-322°C, C_{15}H_{10}O_6 [M^+] 286.0478 was found to be a flavone on the basis of positive Shinoda test and UV spectrum. It showed bands for hydroxyl, conjugated carbonyl and aromatic nucleus in the IR spectrum. On treatment with acetic anhydride and trimethylamine at room temperature, Zr-7 furnished an acetate, m.p. 225-228°C, C_{23}H_{18}O_{10} [HRMS: m/z 454.0900 [M^+]] which exhibited four acetate groups in $^1$H NMR at δ 2.32 (3H, s, 1x OAc), 2.33 (6H, s, 2x OAc), 2.43 (3H, s, 1x OAc).

Compound Zr-7 was characterised as 5,7,3’,4’-tetra hydroxy flavone i.e. luteolin by chemical and spectral data and direct comparison with authentic sample. Zr-7 have not been earlier reported from Z. rugosa.
Zr-8

Compound Zr-8 as yellow granules, m.p. 184-186°C, C_{12}H_{20}O_{11}(M-glucose m/z 286) was found to be a flavonoid on the basis of positive Shinoda test and UV spectrum. It showed bands for hydroxyl, conjugated carbonyl and aromatic nucleus in IR spectrum. Due to high oxygen contents and solubility in water Zr-8 was considered to be a glycoside. The presence of 6 more carbon than 15 carbons of a flavonoid unit as well as the presence of an anomeric hydrogen in $^1$H NMR indicated Zr-8 to be a glycoside of a flavonoid.

Hydrolysis of Zr-8

Zr-8 was refluxed with 6% aqueous hydrochloric acid in MeOH for 4 hours which furnished an aglycone and a sugar residue. The sugar residue was identified as luteolin by paper chromatographic comparison with different known sugars. The aglycone was isolated as yellow crystals, C_{15}H_{10}O_{6} (M^+, 286.0478), m.p. 322-325°C. The aglycone was identified as luteolin by comparison from its spectral data with the reported data and also by direct comparison (co-TLC and mmp) with authentic sample. The attachment of the glucose to luteolin was proved by a study of the UV spectrum with the shift reagent.
Compound Zr-8 was characterised as luteolin-7-0-glucoside. The identity of Zr-8 with luteolin-7-0-glucoside was finally confirmed by its direct comparison (co-TLC, mmp) with an authentic sample. This is the first report of this compound in Z. rugosa.