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ALKALOIDS FROM RHAMNACEAE—XXVI[1]

NUMMULARINE -D, -E AND -F, NEW CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS NUMMULARIA*

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Abstract—From the benzene extract of the root bark of *Zizyphus nummularia* three new peptide alkaloids have been isolated: nummularine-D 1, -E 3 and -F 5. All three contain the 14-membered ring system. Nummularine-D is N-desmethyl-integerrenine. Nummularine-E has *N,N*-dimethylthreonine and nummularine-F has *N,N*-dimethylglycine as terminal amino-acid, neither of which has been found in such alkaloids before.

Recently we described the isolation and characterization of five alkaloids with the 13-membered ring system (nummularine-A, -B, -C, amphibine-H and mucronine-D) from the root bark of *Z. nummularia*.[1] Extensive chromatography of the same extract furnished nine more fractions, the major seven of which were further investigated and found to contain only 14-membered ring peptide alkaloids. Four compounds were identified as frangufoline,[2] amphibine-A,[3] integerrenine[4] and mauritine-F.[5] The other three previously unknown alkaloids **1**, **3** and **5** will be described here.

Nummulairine-D **1** (*N-desmethyl-integerrenine*)

The chromatographic behaviour of this alkaloid was nearly identical with that of integerrenine. The IR spectrum displayed characteristic secondary amid bands, conjugated double bond, an aryl ether and *N*-methyl groups. The UV spectrum showed only end absorption which revealed the presence of a 14-membered ring system.[6] The PMR spectrum confirmed the presence of one *N*-methyl group and a cis-styrylamine moiety.

The mass spectrum of **1** (Fig. 1) closely resembled that of integerrenine,[4,7] the only difference consisting in a downward shift of the peaks M^+, **a** and **b** by 14 mass units, suggesting that **1** is *N*-desmethyl-integerrenine. More evidence of the postulated structure was achieved by transforming **1** into its *N*-acetyl derivative **2**, which exhibited in its PMR spectrum at δ1·82 and 2·4 singlets for a *N*-acetyl and for one *N*-methyl group. Finally **1** was submitted to a reductive *N*-methylation reaction.[8] The obtained product was found to be identical in m.p., IR, mass spectrometric and chromatographic behaviour with dihydrointegerrenine,[4] thus proving the structure of **1**.

Nummularine-E **3**

Isolated from the polar fraction by thin layer chromatography on silica gel plates, the alkaloid was found to be sparingly soluble in most of common organic solvents and did not give the usual Dragendorff reaction for alkaloids. The IR spectrum of **3** in KBr was, however, typical of peptide alkaloids and showed strong bands characteristic of hydroxyl, secondary amide, *N*-methyl, conjugated double bond and phenol ether groups. Its UV spectrum showed only strong end absorption, characteristic of styrylamine chromophore in the 14-membered ring containing alkaloids.

The mass spectrum of **3** (Fig. 2) was most revealing and allowed a unique structure to be assigned to it. The principal fragments whose elementary compositions were measured by high resolution mass spectrometry are described in chart. (1) α-Cleavage at the terminal amino acid led to the base peak at *m/e* 102, which was shifted in acetyl-nummularine-E **4** to *m/e* 144, thus proving the presence of a hydroxy-amino acid. The PMR spectrum of **3** in DMSO-solution showed at δ1·65 a singlet for the *N,N*-dimethylamino group and at δ1·23 at doublet (J = 5 cps), which could be assigned to the *C*-methyl group of *N,N*-dimethyl-threonine by comparison with the PMR spectrum of synthetic methyl *N,N*-dimethyl-threoninate. In conformity with the presence of a free hydroxyl group in the molecule, an important primary mass spectrometric decomposition arising from a McLafferty rearrangement[9-11] with transfer of a hydrogen atom from the

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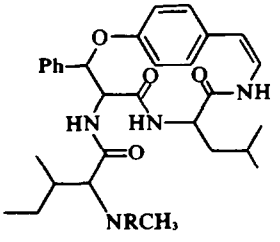
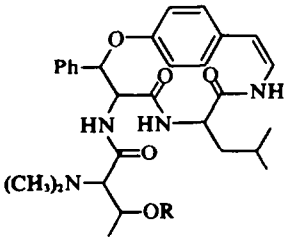


1: R = H
2: R = $COCH_3$



3: R = H
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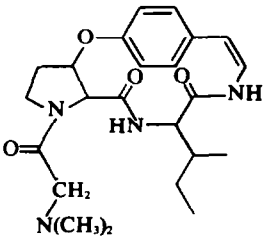


5

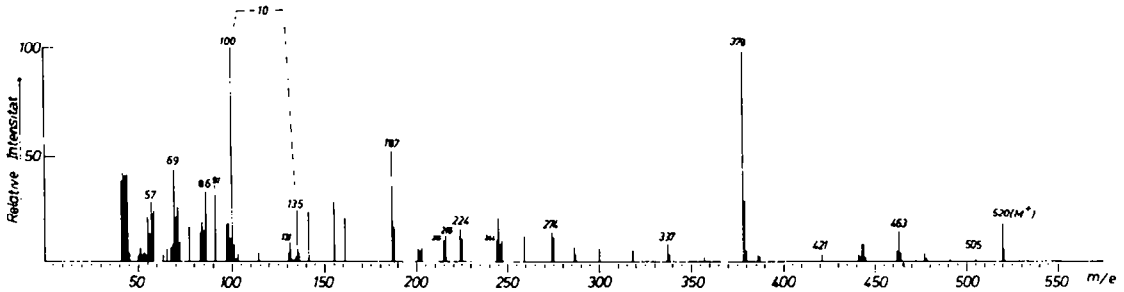


Fig. 1.

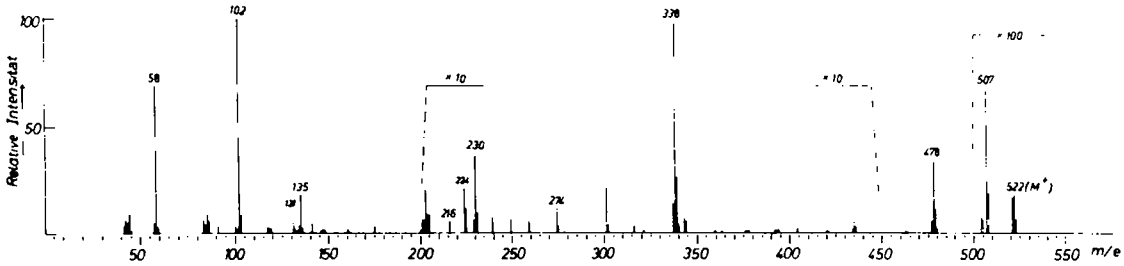
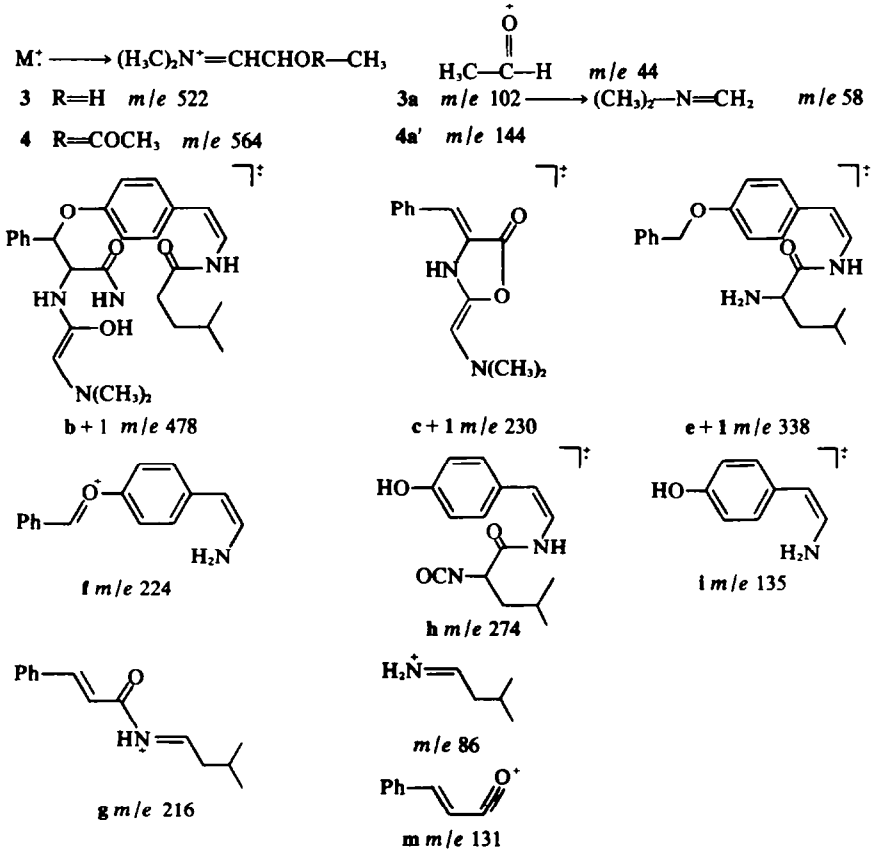


Fig. 2.

OH-group to a neighbouring carbonyl group, thus yielding the peak **b** + 1 (*m/e* 478) and to a smaller extent ionized acetaldehyde (*m/e* 44). As a consequence of hydrogen transfer, the peaks **c** and **e** are now shifted to **c** + 1 and **e** + 1.[7]

The presence of β-phenylserine in the molecule was established by the fragments **b** + 1, **c** + 1, **e** + 1, **f**, **m** and by the PMR spectrum of 3 in trifluoroacetic acid, which showed for the absorption of the *N,N*-dimethylamino group a downfield shift to a pair of badly resolved signals at δ2·18 and 2·72, behaviour characteristic of integerrenine-type alkaloids.[4] That the other amino-acid present could be leucine or isoleucine was indicated by the fragments **e** + 1, **h**, **g**, and the amine fragment at *m/e* 86. That indeed leucine was present was shown by acid hydrolysis of dihydro-nummularine-E and the comparison of the hydrolysate with authentic samples on paper chromatography.[12,13] Thus, structure 3 is established for nummularine-E. It appears that this compound is the first cyclopeptide alkaloid carrying *N,N*-dimethyl-threonine as the terminal amino-acid. Its stereochemistry in 3, however, has still to be established.



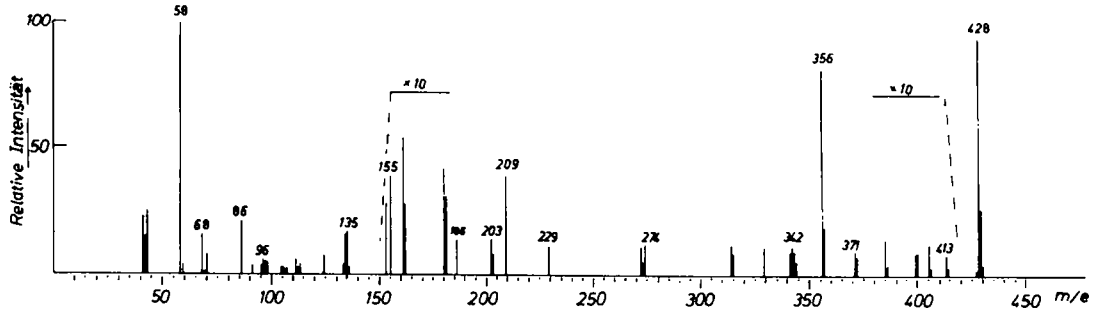


Fig. 3.

Nummularine-F **5**

By high resolution mass spectrometry the elementary composition of **5** was determined as $C_{23}H_{32}N_4O_4$. The IR spectrum showed the usual secondary amide and conjugated C=C double bond absorption, plus bands attributable to phenolic ether and *N*-methyl groups. The UV spectrum revealed the presence of a 14-membered ring system. The PMR spectrum showed at δ2·23 a singlet for a *N,N*-dimethylamino group and at δ3·05 a singlet which, by comparison with synthetic *N,N*-dimethylglycine, could be attributed to the methylene group of the terminal amino acid.

The mass spectrometric fragmentation of **5** (Fig. 3) closely follows the previously-described schemes for the structurally similar alkaloids amphibine-F, -G[14] and mauritine-F.[5] To confirm the assignment of the fragment ions, extensive high resolution measurements were made. Characteristic peaks in the lower mass range indicate the presence of *N,N*-dimethylglycine (**a**, *m/e* 58, $CH_2{=}\overset{+}{N}(CH_3)_2$), hydroxyproline (*m/e* 96, $C_5H_6NO^+$ and *m/e* 68, $C_4H_6N^+$), p-hydroxystyrylamine (*m/e* 135, HO–C_6H_4–CH=CH–NH_2^+) and a C_6 amino acid (*m/e* 86, C_4H_9–$CH{=}\overset{+}{N}H_2$). The fragment **k'** (*m/e* 155, $C_8H_{15}N_2O^+$) indicates that *N,N*-dimethylglycine is attached to hydroxyproline. The p-hydroxystyrylamine group is joined to hydroxyproline by an ether linkage, as can be deduced from the peaks **r** (*m/e* 229, $C_{13}H_{13}N_2O_2^+$), **s** (*m/e* 203, $C_{12}H_{15}N_2O^+$), **t** (*m/e* 186, $C_{12}H_{12}NO^+$) and it is also joined to a C_6 amino acid by an amide bonding as can be seen from the fragment **u** (*m/e* 274, $C_{15}H_{18}N_2O_3^+$). The peaks **p** (*m/e* 209, $C_{11}H_{17}N_2O_2^+$) and **g** (*m/e* 181, $C_{10}H_{17}N_2O^+$) demonstrate the direct bonding between hydroxyproline and the leucine isomer, thus confirming the structure of the complete ring system.

Upon acid hydrolysis of dihydronummularine-F which was obtained from **5** by catalytic hydrogenation, *N,N*-dimethylglycine and isoleucine were identified by paper chromatography. The structure is therefore represented by formula **5**.

EXPERIMENTAL

M.ps were determined on a Kofler microscope stage, optical rotations were measured using a Perkin–Elmer 141 photoelectric polarimeter. Cary 14 (UV) and Perkin–Elmer 221 (IR) spectrophotometers were used, and PMR studies were carried out with Varian A-60. Mass spectral analyses were performed on an AEI MS-9 mass spectrometer operating at 70 eV with evaporation of the samples in the ion source at about 200°. TLC, unless otherwise specified, was carried out on silica gel Merck 60 $PF_{254+366}$.

Extraction of Z. nummularia. The crude alkaloids (20 g) were obtained by extraction of the powdered bark (10 kg) in the usual manner.[15] The mixture of crude alkaloids was fractionated on 2 kg silica gel M(Gebr. Herrmann/Köln) column, eluting with increasingly polar CH_2Cl_2–MeOH mixtures, into 11 fractions. The chromatographic separation was followed using an LKB Uvicord, and the collected fractions were analysed by TLC, proving in every case to be mixtures of two or three main components. These fractions were separated into individual components using preparative TLC or column chromatography.

Frangufoline (0·7 g), amphibine-A (0·3 g), integerrenine (0·08 g), mauritine-F (0·8 g) nummularine-A (1·1 g), -B (2·0 g) and -C (0·3 g), amphibine-H (0·15 g and mucronine-D (0·38 g) were identified by spectroscopic methods and by chromatographic comparison of the compounds with known alkaloids in several solvent systems.

Nummularine-D **1**. 25 mg of **1** were obtained from fraction II by repetitive chromatography on silica gel using cyclohexane–Me_2CO–MeOH (35:15:1) as solvent system, m.p. 265–68° (MeOH) $[\alpha]_D^{20}$ −186° (c 0·2, $CHCl_3$) $\nu_{max}^{CHCl_3}$ 3345, 1670 (secondary amide); 2745 (N-Me); 1630 (C=C); 1250, 1030 (aryl ether, λ^{MeOH} strong end absorption only. δ^{CDCl_3} 0·65–9·95 signal complex (12 H, 4 C-Me); 2·18 s (3 H, N-Me); 6·35 d J = 8 Hz (1H, vinyl H); 6·4–7·5 signal complex (13H, 9ArH, 1 vinyl H and 3 NH). Mol. wt. (MS) 520·3051; calcd. for $C_{30}H_{40}N_4O_4$, 520·3050.

N-Acetyl-nunnularine-D **2**. 8 mg of **1** were dissolved in 1 ml pyridine and 1 ml Ac_2O was added. The mixture was left overnight at room temp. After that EtOH was added and the excess of Ac_2O and pyridine was removed under reduced pressure. **2** was purified by TLC (cyclohexane–Me_2CO 3:2) followed by recristallization, m.p. 210–13° ($CHCl_3$-petroleum ether). $[\alpha]_D^{20}$ −296° (c = 0·2 $CHCl_3$). δ^{CDCl_3} 0·68–0·85 (12 H, 4 C-Me); 1·82 s (3H, N-Ac); 2·4 s (3H, N-Me); 6·41 d J = 9 Hz (1H, vinyl H); 7·1–8·0 signal complex (12H, 9 ArH, 1 vinyl H and 2 NH). Mol. wt. (MS) 562.

N-Methyldihydronummularine-D. 5 mg of **1** were dissolved in 15 ml MeOH, then 1 ml HCOOH and 10 mg 10% Pd-C were added. After 9 hr at room temp in a hydrogen atmosphere, and flushing with nitrogen, the catalyst was filtered off and the filtrate was evaporated to yield N-methyl dihydronummularine-D, m.p. 314–16° (MeOH). $[\alpha]_D^{20}$ −48·1° (c 0·5, $CHCl_3$), which proved to be identical with dihydrointegerrenine.[4]

Nummularine-E **3**. 1·1 g of this alkaloid were separated from fraction V by TLC using CH_2Cl_2–Me_2CO–MeOH (35:15:1) as a solvent system, m.p. 278–79° (MeOH). $[\alpha]_D^{20}$ +12° (c 0·02 MeOH), ν_{max}^{KBr} 3380, 3260 (OH, NH); 2775 (NMe); 1680 (amide); 1625 (conjugated C=C); 1220, 1030 (aryl ether), λ^{MeOH} end absorption. δ^{DMSO} 1·23 d J = 5 Hz (3H, $CHOHC\underline{H}_3$); 1·65 s (6H, NMe_2), δ^{CF_3COOH} 0·73–1·1 signal complex (6H, 2 C-Me); 1·23 d J = 7 Hz (3H, $CHOHC\underline{H}_3$); 2·18 (3H, NMe_2); 2·72 (3H, NMe_2); 6·04 d J = 9 Hz (1H, vinyl H); 6·8–8·4 (13 H, 9 ArH, 1 vinyl H, 3 NH). Mol. wt. (MS) 522·2834; calcd. for $C_{29}H_{38}N_4O_5$, 522·2824.

O-Acetyl-nummularine-E **4**. 10 mg of **3** were acetylated with Ac_2O in pyridine as described before, m.p. 230–32° ($CHCl_3$). $\nu_{max}^{CHCl_3}$ 3335 (NH); 2760 (NMe); 1720 ($OCOCH_3$); 1665 (amide); 1610 conjugated C=C); 1215, 1030 (aryl ether); Mol. wt. (MS) 564.

Dihydronummularine-E. 35 mg of **3** were dissolved in 30 ml MeOH and 20 mg 10% Pd-C were added and kept at room temperature in H_2. After 6 hr the catalyst was filtered off and the filtrate was evaporated down to yield the dihydro derivative, m.p. 282° (MeOH). $[\alpha]_D^{20}$ +14° (c 0·02 MeOH); Mol. wt. (MS) 524.

Hydrolysis of dihydronummularine-E. 10 mg of dihydronummularin-E were heated in a sealed tube with 1 ml 6N

HCl for 24 hr at 110°. The excess reagent was evaporated *in vacuo* and the residue was taken up with water for paper chromatography and TLC. *N,N*-Dimethylthreonine and isoleucine were identified by comparison with synthetic specimen on Whatman No. 1 paper using n-BuOH-AcOH-H_2O (4:1:5)[12] and n-BuOH sat with pH 4 citric acid buffer,[13] and ninhydrin and iodine as spray reagents.

Nummularine-F **5**. 50 mg of **5** were obtained from fraction VII using C_6H_6-AcOEt-MeOH (25:15:4) as a solvent system, m.p. 120° (MeOH); $[\alpha]_D^{20}$ −204° (c 0·2 MeOH); $\nu_{max}^{CHCl_3}$ 3380, 1675 (secondary amide), 2790 (N–Me), 1620 (conjugated C=C), 1225, 1030 (aryl ether; λ^{MeOH} only strong end absorption. δ^{CDCl_3} 0·8–0·95 signal complex (6H, 2 C-Me), 2·21 s (6H, N-Me_2), 3·05 s (2H, $COCH_2N$), 4·21 d J = 6 Hz (1H, HyPro-2-H), 5·45 m (1H, HyPro-3-H), 6·4–7·4 (7H, 4 ArH, 1 vinyl H and 2NH), Mol. wt. (MA) 428·2429; calcd. for $C_{23}H_{32}N_4O_4$ 428·2424.

Dihydronummularine-F was prepared according to the method described before, m.p. 175° (MeOH); $[\alpha]_D^{20}$ −159° (c 0·23 MeOH). Mol. wt. (MS) 430.

Hydrolysis of dihydronummularine-F. The reaction was carried out as described above. *N,N*-Dimethylglycine and isoleucine were identified by comparison with synthetic materials on Whatman No. 1 paper and on cellulose plates using n-BuOH sat with pH 4 citric acid buffer and n-BuOH-AcOH-H_2O (4:1:5) as solvent systems.

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