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CYCLOPEPTIDE ALKALOIDS OF *ZIZYPHUS OENOPLIA*[1]

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Abstract—The known alkaloids zizyphine-A (1) and -B (2), abyssinine-A (4) and -B (5), and the previously undescribed zizyphine-C (3), -D (6) and -E (7) have been isolated from the stem bark of *Zizyphus oenoplia*. The structures of the new compounds were elucidated by spectroscopic methods applied to the alkaloids and to some of their transformation products, and by chemical degradation reactions.

While working up the crude bases obtained from the stem bark of *Zizyphus oenoplia* Mill.† for a revision of the structure of zizyphine-A (1),[2] the mixture was found to contain, besides this substance and the previously described zizyphine-B (zizyphinine) (2),[2,3] several other alkaloids, some of which are now reported. Extensive chromatography of the crude bases furnished eight apparently homogeneous fractions (Table 1), one of which was subsequently shown to be composed of two substances separable only by chemical means. Of these nine alkaloids, the structures of four are already known: zizyphine-A and -B, and abyssinine-A (4) and -B (5).[1] Two of the so far undescribed substances were present in insufficient amounts for structural work to be carried out. Of the remaining three, one, zizyphine-C (3) was found to contain a 13-membered ring like zizyphine-A and -B. The other two new compounds, zizyphine-D (6) and -E (7), have 15-membered rings like abyssinine-A and -B, with the peculiarity that one of the amino acids is 3-hydroxy-isoleucine.

Zizyphine-C (3). The mass spectrum of this alkaloid, which shows a molecular ion peak at *m/e* 645 and an intense base peak at *m/e* 148, closely resembles the spectrum of zizyphine-A, with most of the peaks displaced 34 mass units upwards, suggesting that zizyphine-C is an analog of zizyphine-A with the terminal N,N-dimethyl-isoleucine moiety replaced by an N,N-dimethylphenylalanine residue.

The IR spectrum displays characteristic secondary amide bands as well as absorption attributable to a conjugated, *cis*-1,2-disubstituted C=C double bond, an aryl ether, methoxy and N-Me groups. The UV spectrum shows that a fairly flat styrylamine chromophore is present, as in the cases of zizyphine-A and -B, mucronine-D,[4] and amphibine-H.[5]

The PMR spectrum confirms the presence of an N,N-dimethyl group, a OMe group and a *cis*-styrylamine moiety. Two NH signals are recognizable, one of them partly obscured by the large singlet due to phenyl protons. As in the cases of zizyphine-A and amphibines-B, -C, -D and -E,[6] the coupling (J = 6 Hz) between the protons at C-2 and C-3 of the 3-hydroxyproline unit (δ 4·33 and 5·24, respectively) indicates that the dihedral angle between them is probably in the ranges 45–55° or 130–140°.[7]

Oxidative cleavage of the styrene double bond with $OsO_4/NaIO_4$ yielded a single product which was

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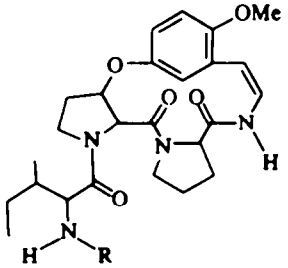
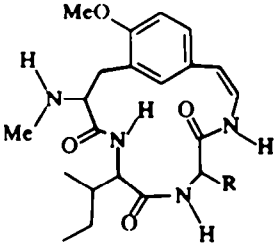
Table 1. Chromatographically homogeneous alkaloidal fractions from *Zizyphus oenoplia* Mill

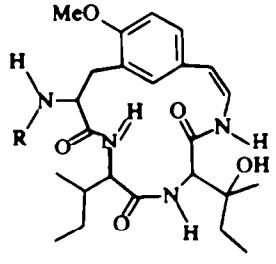
Fraction	Percentage of crude alkaloids	Composition	M⁺	Base peak
I	52·5	zizyphine-A	611	114
II	7·6	zizyphine-B and -C (1:2)	597, 645	100, 148
III	7·6	zizyphine-D	474	72
IV	4·9	abyssinine-B	458	163
V	4·0	zizyphine-E	460	72
VI	1·5	—	701	114
VII	0·8	abyssinine-A	458	163
VIII	0·6	—	486	72



1: R = N,N-dimethyl-Ile
2: R = N-methyl-Ile
2a: R = N-acetyl-N-methyl-Ile
3: R = N,N-dimethyl-Phe



4: R = i-Bu
5: R = s-Bu



6: R = Me
7: R = H

shown mass-spectrometrically to contain all the amino acid residues and the methoxylated aromatic moiety of the original alkaloid, proving the macrocyclic nature of zizyphine-C. The amino acid sequence can also be deduced from the mass spectrum of this compound, which again is very similar to that of the amido-aldehyde derived from zizyphine-A.[3] The UV spectrum of the oxidation product of zizyphine-C resembles that of 2,5-dimethoxybenzaldehyde,[9] showing that the styrylamine substituents occupy the 2- and 5-positions. As the UV, PMR and mass spectra of zizyphine-C can be clearly related to those of the other cyclopeptide alkaloids with a 13-membered ring, the OMe group must be attached to the 2-position, and the 3-hydroxyproline unit to the 5-position. The isolation of 2-(5-hydroxy-2-methoxyphenyl)ethylamine[5] from the basic hydrolysate of hydrogenated zizyphine-C proves that the substitution pattern on the styrylamine residue is the same as in the previously described 13-membered ring cyclopeptide alkaloids.[4,2]

The PMR spectrum of the oxidation product shows the presence of a single formyl group, indicating that the initial cleavage product has lost its N-CHO group in the process of isolation. As with the oxidation product of zizyphine-A, no coupling is any longer directly observable between the protons at C-2 and C-3 of the 3-hydroxyproline residue. As the coupling constants for the C-2 and C-3 protons of *cis*- and *trans*-3-hydroxyproline are 4·0 and 1·0, respectively,[6] the *trans* configuration of the 3-hydroxyproline moiety seems to be more likely on this spectrometric basis. An unusual feature in this spectrum (in $CDCl_3$) is the presence of two distinct N-Me resonances for the NMe_2 group.

Ozonolysis of zizyphine-C, followed by acid hydrolysis, made the chromatographic identification of 3-hydroxyproline possible, as well as that of proline and isoleucine. Upon acid hydrolysis of the alkaloid with no previous treatment, proline, isoleucine, and N,N-dimethylphenylalanine were identified. The complete structure is therefore represented by formula 3.

Zizyphine-D and -E (**6** *and* **7**). Due to the presence of a 3-hydroxyisoleucine residue, the mass spectra of zizyphine-D and -E differ significantly from those of the 15-membered ring cyclopeptide alkaloids known so far.[1,9] Zizyphine-E is rather sensitive to thermal decomposition, but at 160° it gives a distinct molecular ion peak at *m/e* 460, while zizyphine-D gives a molecular ion peak at *m/e* 474 under the usual conditions. As observed with other cyclopeptide alkaloids containing a hydroxy-amino acid residue,[10-12] the most important primary decomposition is a McLafferty rearrangement with transfer of a H atom from the OH-group to a neighbouring CO group, leading in the present cases to the fragments $C_4H_8O^+$ (*m/e* 72) and (M-72)$^+$. Consecutive α-cleavage of the (M-72)$^+$ ions at the amino group, and elimination of isocyanic acid, yield the radical-ions a', which in a characteristic secondary reaction split off a Me or an Et radical, thus proving the presence of an isoleucine unit. In the lower mass range of both spectra, the typical fragments of primary and secondary 15-membered ring cyclopeptide alkaloids are recognizable,[9] and additionally, the intense peaks at *m/e* 43 and *m/e* 57 originating from $C_4H_9O^+$. The elemental composition of all fragments was confirmed by high-resolution measurements, and the cleavage sequence was substantiated by metastable ion defocusing experiments.

The IR spectra of zizyphine-D and -E show the usual secondary amide and conjugated C=C double bond absorptions, plus bands attributable to

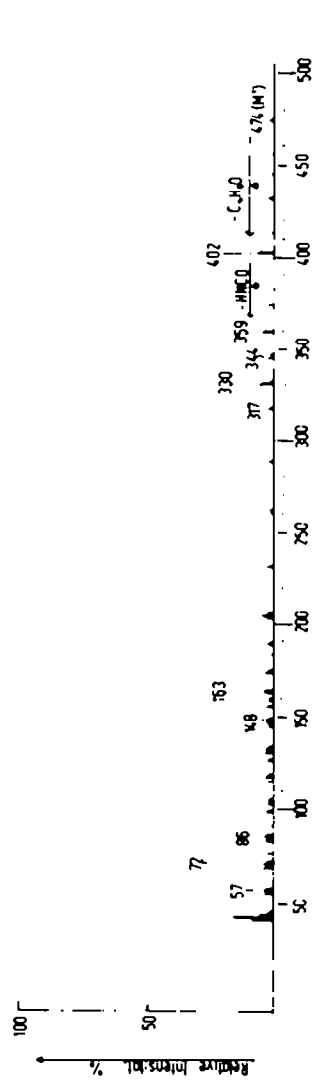


Fig 1. Mass spectrum of zizyphine-D (6).

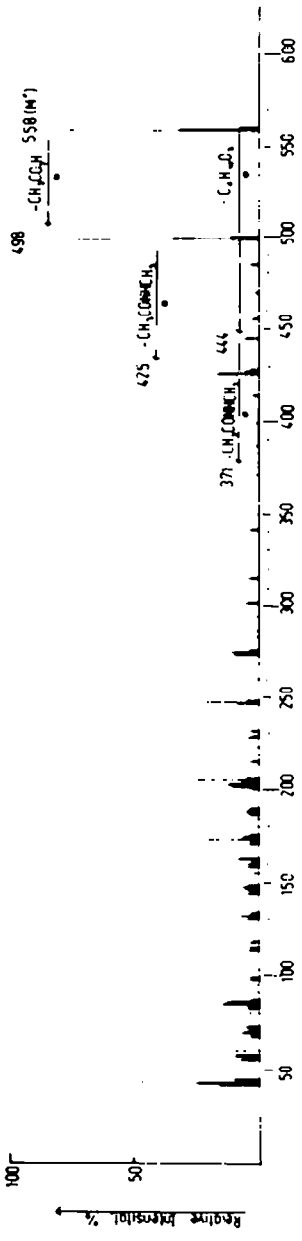


Fig 2. Mass spectrum of N,O-diacetyl-zizyphine-D.

phenolic ether, OMe and, in the case of zizyphine-D, N-Me groups. The UV spectra, which display a single intense maximum at 274 nm, resemble those of other 15-membered ring cyclopeptide alkaloids.[1,9]

The PMR spectra of both alkaloids are remarkably similar, with the presence of a signal due to an N-Me group in the spectrum of zizyphine-D as the only noteworthy difference. An O-Me group, a *cis*-styrylamine moiety and two further NH resonances are easily recognizable. An isolated C-Me singlet at a lower field than the rest of the C-Me signal complex corresponds to the 3-hydroxyisoleucine residue.

The N-acetyl and N,O-diacetyl derivatives of zizyphine-D and -E were prepared and studied spectrometrically. The mass spectra of the N-acetyl derivatives, like those of the parent alkaloids, show weak but distinct molecular ion peaks, fragmentation being characterized by the initial loss of butanone. The following step, as with the N-acetyl derivatives of the abyssinines,[1] is the elimination of acetamide or N-methylacetamide. The mass spectra of the N,O-diacetyl derivatives are characterized by very intense molecular ion peaks. The main fragmentation process is initiated by the loss of acetic acid, followed by elimination of acetamide or N-methylacetamide. A less important route involves the loss of the whole side chain bearing the acetic ester group, followed again by elimination of acetamide or N-methylacetamide. High-resolution measurements and metastable ion defocusing experiments were used to confirm the elemental composition of all fragments and the cleavage sequence. The PMR spectra confirm the presence of N- and O-acetyl groups in these derivatives.

The product isolated after oxidative cleavage of N-acetyl-zizyphine-D is the imido-aldehyde, as indicated by its mass and PMR spectra. The loss of the usual *m/e* 72 fragment is again apparent in the mass spectrum of this substance. The UV spectrum shows only one maximum at 274 nm, indicating that the single methoxy group occupies the *para* position regarding the aldehyde function.[9]

Acid hydrolysis of zizyphine-D allowed the identification of isoleucine and 3-hydroxyisoleucine. Its structure is therefore represented by formula **6**, and that of zizyphine-E, its N-nor-homologue, by formula **7**.

Microbiological tests. Zizyphine-A, N-acetyl-zizyphine-B, zizyphine-C, zizyphine-D and -E and their N-acetyl and N,O-diacetyl derivatives were screened as possible growth inhibitors for bacteria and fungi (*Escherichia coli*, *Bacillus subtilis*, *Pythium debaryanum*, and *Trichoderma viride*). No antibiotic activity was observed.

EXPERIMENTAL

M.ps were determined on a Kofler microscope stage, and 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 and Bruker-Physik HX-90 spectrometers. Mass spectral analyses were performed on an A.E.I. 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 HF_{254} or 60 $PF_{254+366}$ (Merck) using cyclohexane–acetone (1:2) (System I) or $CHCl_3$–EtOH (87·5:12·5) (System II). The crude bases were isolated as described previously,[4] in 0·05% yield.

Chromatographic separation of the alkaloids. The mixture of crude bases was fractionated, 2·5 g at a time, on 500 g silica gel M (Gebr. Herrmann/Köln) column, eluting with increasingly polar CH_2Cl_2–MeOH mixtures. The chromatographic separation was followed using an LKB Uvicord, and the collected fractions were analysed by TLC, proving to be in every case mixtures of two or three main components. These were separated by preparative TLC or dry-column chromatography using system I, yielding the chromatographically homogeneous fractions shown in Table 1. Except for Fraction II, all were shown to be reasonably pure by mass spectrometry.

Identification of zizyphine-A and abyssinine-A and -B. The mass spectrum of Fraction I led to this substance's identification with zizyphine-A isolated previously,[2] which was confirmed by comparative TLC using Systems I and II and, in addition, $CHCl_3$–acetone (68:32), CH_2Cl_2–MeOH (48:2), $CHCl_3$–CH_3CN–MeOH (21:9:1), and C_6H_6–AcOEt–EtOH (5:10:1).

Fractions VII and IV were identified by the same methods with abyssinine-A and -B, respectively, isolated from *Zizyphus abyssinica*.[1]

Separation of zizyphine-C and N-acetyl-zizyphine-B. The MS of Fraction II indicated the presence of two substances, with probable molecular ions at *m/e* 645 and 597, and base peaks at *m/e* 148 and 100. On the hypothesis that the mixture might contain "zizyphinine", the chromatographic systems described in the original publication[10] were tried without success. Two substances could be distinguished using TLC on silica-gel with butanone–pyridine–H_2O–AcOH (70:15:15:2), but the system proved inadequate for prepatative work.

The mixture was acetylated with Ac_2O in pyridine, and the products were separated into two main fractions using System II. The major one, about 2/3 of the total, proved to be the substance with M^+ at *m/e* 645 and base peak at *m/e* 148, here referred to as zizyphine-C. The minor component was shown to be N-acetyl-zizyphine-B.

N-Acetyl-zizyphine-B (**2a**). Amorphous, $[\alpha]_D^{20} - 383 \pm 6°$ ($c = 0{\cdot}10$ $CHCl_3$), $-371 \pm 6°$ ($c = 0{\cdot}10$ MeOH). $\bar{\nu}_{max}^{CHCl_3}$ 3380, 1675, 1640 (secondary amide); 1597, 690 (conjugated *cis*-1,2-disubstituted C=C); 1260, 1050 cm^{-1} (aryl ether). λ_{max}^{MeOH} (log ϵ) 264 (3·76), 318 nm (3·55). δ^{CDCl_3} 0·75–1·00 signal complex (12 H, 4 C-CH_3); 2·12 s (3 H, N-Me); 3·81 s (3 H, O-Me); 5·29 m (1 H, 3-HyPro-3-H); 5·96, 6·63, 8·36 ABX system $J_{AB} = 8{\cdot}6$ Hz, $J_{BX} = 11{\cdot}4$ Hz (vinylamine); 6·46–7·26 signal complex (5 H, 3 ArH + 1 NH + 1 vinyl H). Mol. wt. (MS) 639·3638; calcd. for $C_{34}H_{49}N_5O_7$, 639·3632.

Zizyphine-C (**3**). Amorphous, $[\alpha]_D^{20} - 331 \pm 5°$ ($c = 0{\cdot}10$ $CHCl_3$), $-343 \pm 5°$ ($c = 0{\cdot}10$ MeOH). $\bar{\nu}_{max}^{CHCl_3}$ 3400, 1690, 1645 (secondary amide); 1597, 690 (conjugated *cis*-1,2-disubstituted C=C); 1257 (aryl ether), 2820 (O-Me), 2785 cm^{-1} (N-Me). λ_{max}^{MeOH} (log ϵ) 266 (3·88), 318 nm (3·74). δ^{CDCl_3} 0·80–1·05 signal complex (12 H, 4 C-Me); 2·36 s (6 H, 2 N-Me); 3·80 s (3 H, O-Me); 4·33 d, $J = 6{\cdot}0$ Hz (1 H, 3-HyPro-2-H); 5·24 m (1 H, 3-HyPro-3-H); 5·95, 6·92, 8·37

ABX system J_{AB} = 8·9 Hz, J_{BX} = 11·4 Hz (vinylamine); 7·25 s (5 H, C_6H_5). Mol. wt. (MS) 645·3524; calcd. for $C_{36}H_{47}N_3O_6$, 645·3526.

Oxidation of zizyphine-C. 200 mg zizyphine-C and 5·0 mg OsO_4 were dissolved in 40 ml acetone and 30 ml H_2O, and 140 mg $NaIO_4$ were added in small portions, stirring all the time, in the course of 0·5 h. The solution was stirred during 3 h more, concentrated to approximately 30 ml, and extracted with CH_2Cl_2. The residue of the CH_2Cl_2 extract was chromatographed using System II, yielding 43 mg of the pure amido-aldehyde, $[\alpha]_D^{20}$ −64 ± 1° (*c* = 0·10 $CHCl_3$), −51 ± 1° (*c* = 0·10 MeOH). λ_{max}^{MeOH} (log ε) 212 (shoulder) (4·60), 255 (3·81), 346 nm (3·46). δ^{CDCl_3} 2·31 s (3 H, N-Me); 2·41 s (3 H, N-Me); 3·91 s (3 H, O-Me); 7·41 bs (2 H, NH_2); 8·63 J = 8·2 Hz (1 H, NH); 10·47 s (1 H, CHO). Mol. wt. (MS) 649; calcd. for $C_{35}H_{47}N_3O_7$, 649. (M-C_5H_5) 558·2903; calcd. for $C_{29}H_{40}N_3O_7$, 558·2927.

Oxidation of zizyphine-A. 200 mg zizyphine-A and 6·1 mg OsO_4 were submitted to the same treatment as zizyphine-C. 58 mg of pure imido-aldehyde were isolated, $[\alpha]_D^{20}$ −89 ± 2° (*c* = 0·10 $CHCl_3$), −93 ± 2° (*c* = 0·10 MeOH), λ_{max}^{MeOH} (log ε) 214 (shoulder) (4·59), 256 (3·88), 338 nm (3·44), δ^{CDCl_3} 2·32 s (6 H, NMe_2); 3·93 s (3 H, O-Me); 8·0 b (1 H, NH); 9·14 d J = 9·0 Hz (1 H, N-CHO); 10·44 s (1 H, CHO); 10·94 bd (1 H, NH). MS in accordance with the literature.[2]

2-(5-Hydroxy-2-methoxyphenyl)ethylamine. 64·5 mg zizyphine-C were hydrogenated over 10% Pd/C in MeOH during 6 h. The product was dissolved in 2·5 ml EtOH, an equal volume of 50% NaOH was added, and the mixture was refluxed during 24 h, acidified with 6 N HCl, filtered, diluted to 20 ml with sat $NaHCO_3$ aq, and extracted with CH_2Cl_2. The residue obtained upon evaporation of the solvent was identified as 2-(5-hydroxy-2-methoxyphenyl)ethylamine by TLC using n-BuOH–AcOH–H_2O (4:1:1) and by comparison of the IR spectra of this product and of a synthetic sample.[3]

Hydrolysis of zizyphine-C. 10 mg zizyphine-C and 1 ml 6 N HCl were heated in a sealed tube at 110° during 24 h. The excess reagent was evaporated *in vacuo* and the residue taken up with H_2O for paper chromatography and TLC. N,N-dimethylphenylalanine was identified by comparison of a synthetic specimen on Whatman No. 1 paper using n-BuOH—AcOH–H_2O (4:1:5)[14] and n-BuOH sat with pH 4 citric acid buffer,[15] and spraying with Dragendorff's reagent. Isoleucine was tentatively identified using the same system and spraying with ninhydrin reagent. The presence of isoleucine and proline was definitely established in the hydrolysate by two-dimensional TLC of the dansyl derivatives on silica-gel-G using the systems C_6H_6–pyridine–AcOH (40:10:1) and $CHCl_3$–benzyl alcohol–AcOH (70:30:3).[16]

Identification of 3-hydroxyproline. 5 mg zizyphine-C dissolved in 80% formic acid were ozonized at room temperature during 8 h. The solvent was removed *in vacuo*, and the residue was dissolved in 0·5 ml 6 N HCl and heated in a sealed tube at 110° during 24 h. The excess reagent was evaporated and the residue dissolved in H_2O, dansylated and chromatographed bidimensionally with the systems used for the zizyphine-C hydrolysate. 3-Hydroxyproline was identified by comparison with an authentic specimen, as were proline and isoleucine.

Zizyphine-D (**6**), needles, m.p. 195–195° (MeOH–H_2O). $[\alpha]_D^{20}$ +236 ± 4° (*c* = 0·10 $CHCl_3$), −121 ± 2° (*c* = 0·10 MeOH), $\bar{\nu}_{max}^{CHCl_3}$ 3400, 1647, 1670 (secondary amide); 1602, 704 (conjugated *cis*-1,2-disubstituted C=C); 1249, 1030 (aryl ether), λ_{max}^{MeOH} (log ε) 274 nm (4·16). δ^{CDCl_3} 0·80–1·20 signal complex (9 H, 3 C-Me); 1·32 s (3 H, C-Me); 1·60 m (2 H, CH_2); 2·53 s (3 H, N-Me); 3·89 s (3 H, O-Me); 4·51 d J = 8·3 Hz (1 H, amino-acid α-H); 5·67, 6·60, 8·50 ABX system J_{AB} = 9·5 Hz, J_{BX} = 11·4 Hz (vinylamine); 6·58–7·33 signal complex (4 H, 3 ArH and 1 vinyl H); 8·25 bd J = 7·0 Hz (1 H, NH); 9·80 bd J = 8·3 Hz (1 H, NH). Mol. wt. (MS) 474·2842; calcd. for $C_{33}H_{38}N_2O_4$, 474·2842.

N-Acetyl-zizyphine-D. 40-mg zizyphine-D were dissolved in 0·4 ml dry pyridine, 0·2 ml Ac_2O was added, and the mixture was left 6 h at room temp, after which EtOH was added and removed repeatedly under reduced pressure until the smell of pyridine was no longer noticeable. The residue, which contained some starting material and two other substances in very different amounts, was fractionated by TLC using System I. The main product was shown to be N-acetyl-zizyphine-D, $[\alpha]_D^{20}$ −316 ± 5° (*c* = 0·10 $CHCl_3$), −288 ± 5° (*c* = 0·10 MeOH). δ^{CDCl_3} 0·65–1·05 signal complex (9 H, 3 C-Me); 1·15 s (3 H, C-Me); 2·16 s (3 H, N-$COCH_3$); 3·04 s (3 H, N-Me); 3·83 s (3 H, O-Me); 5·75 d J = 9·0 Hz (1 H, styrylamine α-H); 6·65–7·35 signal complex (4 H, 3 ArH and 1 vinyl H); 7·62 bd J = 11 Hz (1 H, NH). Mol. wt. (MS) 516·2944; calcd. for $C_{32}H_{40}N_4O_4$, 516·2947.

N,O-Diacetyl-zizyphine-D. 43 mg zizyphine-D and 1 mg 4-dimethylaminopyridine were dissolved in 4·3 ml dry pyridine, 0·43 ml Ac_2O was added, and the mixture was left 24 h at room temp, after which EtOH was added to remove excess Ac_2O and pyridine as described above. The residue was composed of two main substances, the minor one apparently N-acetyl-zizyphine-D, and was fractionated by TLC using System I. The principal component was shown to be N,O-diacetyl-zizyphine-D, $[\alpha]_D^{20}$ −288 ± 5° (*c* = 0·10 $CHCl_3$), −218 ± 4° (*c* = 0·10 MeOH). δ^{CDCl_3} 0·60–1·05 signal complex (9 H, 3 C-Me); 1·52 s (3 H, C-Me or O-$COCH_3$); 1·61 s (3 H, O-$COCH_3$ or C-Me); 2·20 s (3 H, N-$COCH_3$); 3·05 s (3 H, N-Me); 3·84 s (3 H, O-Me); 5·80, 6·88, 7·49 ABX system J_{AB} = 9 Hz, J_{BX} = 11 Hz (vinylamine); 6·65–7·35 signal complex (4 H, 3 ArH and 1 vinyl H); 8·32 bd J = 9·8 Hz (1 H, NH). Mol. wt. (MS) 558·3064; calcd. for $C_{34}H_{42}N_4O_5$, 558·3054.

Oxidation of N-acetyl-zizyphine-D. 50 mg N-acetyl-zizyphine-D were dissolved in 10 ml acetone and 7·5 ml H_2O, and 3·0 mg OsO_4 were added. In the course of 0·5 h, 44 mg $NaIO_4$ were added in small portions, stirring all the time, and the soln was stirred at room temp during 1·5 h more. Most of the acetone was eliminated by concentrating under reduced pressure to about 10 ml, and the concentrated soln was extracted with CH_2Cl_2. The residue of the CH_2Cl_2 extracts, 54 mg, was chromatographically pure, and was shown to be the expected imido-aldehyde, $[\alpha]_D^{20}$ −139 ± 2° (*c* = 0·10 $CHCl_3$), −65 ± 1° (*c* = 0·10 MeOH). λ_{max}^{MeOH} (log ε) 274 nm (4·08). δ^{CDCl_3} 0·60–1·08 signal complex (9 H, 3 C-Me); 1·35 s (3 H, C-Me); 2·01 s (3 H, N-$COCH_3$); 2·97 s (3 H, N-Me); 3·22 d J = 8·0 Hz (2 H, benzyl-CH_2); 3·93 s (3 H, O-Me); 4·42 m (1 H, amino acid α-H); 4·72 d J = 8·4 Hz (1 H, 3-Hylle α-H); 5·83 m (1 H, amino acid α-H); 6·95 d J = 8·8 Hz (1 H, NH); 7·41 d J = 8·3 Hz (1 H, NH); 7·67–7·83 signal complex (3 H, ArH); 9·10 d J = 9·6 Hz (1 H, N-CHO); 9·82 s (1 H, CHO); 10·35 d J = 9·6 Hz (1 H, NH). Mol. wt. (MS) 548·2851; calcd. for $C_{32}H_{40}N_4O_4$, 548·2846.

Hydrolysis of zizyphine-D. 10 mg zizyphine-D and 1 ml 6 N HCl were heated in a sealed tube at 110° during 24 h. The excess reagent was evaporated under reduced pressure, and the residue was dansylated and submitted to bidimensional TLC on silica-gel-G using C_6H_6–pyridine–AcOH (40:10:1) and $CHCl_3$–benzyl

alcohol–AcOH (70:30:3).[16] Isoleucine and traces of 3-hydroxyisoleucine[17] were identified in the hydrolysate.

Zizyphine-E (7). Amorphous, $[\alpha]_D^{20}$ + 150 ± 2° (*c* = 0·10 $CHCl_3$), − 111 ± 2° (*c* = 0·10 MeOH). $\bar{\nu}_{max}^{CHCl_3}$ 3300, 1673, 1647 (secondary amide); 1602, 704 (conjugated *cis*-1,2-disubstituted C=C); 1250, 1020 (aryl ether); 2820 (O-Me), λ_{max}^{MeOH} (log ε) 279 nm (4·20). δ^{CDCl_3} 0·80–1·20 signal complex (9 H, 3 C-Me); 1·31 s (3 H, C-Me); 1·59 m (2 H, CH_2); 3·89 s (3 H, O-Me); 4·56 d *J* = 8·3 Hz (1 H, amino acid α-H); 5·67, 6·89, 8·50 ABX system J_{AB} = 9·5 Hz, J_{BX} = 11·4 Hz (vinylamine); 6·58–7·20 signal complex (4 H, 3 ArH and 1 vinyl H); 8·32 d *J* = 7·5 Hz (1 H, NH); 9·90 d *J* = 8·9 Hz (1 H, NH). Mol. wt. (MS) 460·2683; calcd. for $C_{24}H_{36}N_4O_5$, 460·2686.

N-Acetyl-zizyphine-E. 44 mg zizyphine-E were dissolved in 4·4 ml dry pyridine, 0·22 ml Ac_2O were added, and the mixture was allowed to react at room temp during 6 h. After elimination of excess reagent and pyridine, the residue was chromatographed (System I) to yield 33 mg pure N-acetyl-zizyphine-E, microcrystalline (MeOH–H_2O), m.p. 155°, $[\alpha]_D^{20}$ + 55 ± 1° (*c* = 0·10 $CHCl_3$), − 210 ± 3° (*c* = 0·10 MeOH), δ^{CDCl_3} 0·70–1·10 signal complex (9 H, 3 C-Me); 1·26 s (3 H, C-Me); 2·02 s (3 H, N-$COCH_3$); 3·82 s (3 H, O-Me); 5·57 d *J* = 9·0 Hz (1 H, styrylamine − H); 6·53–7·17 signal complex (4 H, 3 ArH and 1 vinyl H); 7·62 bd *J* = 7 Hz (1 H, NH); 8·29 bd *J* = 11 Hz (1 H, NH); 8·64 bd *J* = 8 Hz (1 H, NH). Mol. wt. (MS) 502·2783; calcd. for $C_{26}H_{38}N_4O_6$, 502·2791.

N,O-Diacetyl-zizyphine-E. 62 mg zizyphine-E were dissolved with 2·0 mg 4-dimethylaminopyridine in 0·62 ml dry pyridine, and 0·31 ml Ac_2O were added, leaving the soln at room temp during 24 h. Excess Ac_2O and pyridine were removed as before, and the residue was chromatographed (System II) to yield 30 mg pure N,O-diacetyl-zizyphine-E, hexagonal bipyramids and needles from MeOH–H_2O, which sublime above 270° giving very fine needles, infusible up to 350°. $[\alpha]_D^{20}$ − 62 ± 1° (*c* = 0·10 $CHCl_3$), − 64 ± 1° (*c* = 0·10 MeOH). Mol. wt. (MS) 544·2891; calcd. for $C_{28}H_{40}N_4O_7$, 544·2897.

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