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REVISED STRUCTURES FOR THE POLYANDROCARPIDINES

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Abstract The polyandrocarpidines are N-alkyl-γ-alkylidene-γ-lactams (2a,b, 3a,b) rather than amides of a cyclopropenyl acid (1a,b)

In 1972, we reported that crude extracts of the red encrusting tunicate *Polyandrocarpa* (*Eusynstela*) sp showed potent antimicrobial activity [1] In 1978, Cheng and Rinehart proposed that a 9 1 mixture of two unusual cyclopropene derivatives, polyandrocarpidine I (1a) and polyandrocarpidine II (1b) were responsible for the antimicrobial activity [2] Although our ^{1}H NMR data was obviously not compatible with the proposed structures, we have only recently been able to define new structures for the polyandrocarpidines We find that the polyandrocarpidines are γ-methylene-γ-lactams isomeric with the cyclopropene derivatives proposed previously

We have examined several specimens of *Polyandrocarpa* sp [3] The ethyl acetate-soluble material from the methanolic extract of each specimen was first chromatographed on TLC grade silica gel using an eluant gradient from chloroform to methanol, then chromatographed on Sephadex LH-20 using 1 1 methanol-dichloromethane as eluant The resulting antimicrobial fractions (1 4% dry weight) were analyzed by HPLC on C-18 porasil using 25% acetonitrile and 75% 0 05 M aqueous phosphate buffer (pH = 5) containing 0 005 M tetrabutylammonium bisulfate These analyses indicated that the active material was indeed a 9 1 mixture of homologs, as demonstrated previously, but that each homolog was a mixture of isomers with the isomer ratios varying from 4 1 to 1 1 We have therefore designated these compounds as polyandrocarpidines A-D with polyandrocarpidines A (2a), and B (3a) corresponding to polyandrocarpidine I and polyandrocarpidines C (2b) and D (3b) corresponding to polyandrocarpidine II Hydrogenation of each of the polyandrocarpidine mixtures gave the same 9 1 ratio of two hexahydro-polyandrocarpidines 4a,b indicating that polyandrocarpidines A and B (or C and D) were geometrical isomers

The HPLC system used for analysis of the isomer mixtures proved very difficult to use as a preparative system since the tetrabutylammonium bisulfate forms a complex with the polyandrocarpidines making it difficult to recover the pure polyandrocarpidines We therefore obtained ^{13}C NMR spectral data by comparison of the spectrum of a 1 1 mixture of geometrical isomers with that of a 4 1 mixture of geometrical isomers ^{1}H NMR spectral data of polyandrocarpidines A and B were obtained using samples containing small quantities of tetrabutylammonium bisulfate [4]

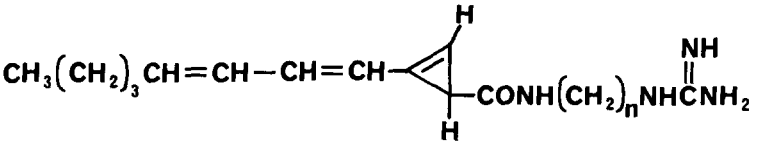
The ^{1}H NMR spectrum[5] of polyandrocarpidine A (2a) contained signals at δ 3 45 (t, 2 H, J = 7 Hz), 3 04 (q, 2 H, J = 7 Hz), 1.65 (m, 4 H) and 1 35 (m, 2 H) due to the $-N-CH_2-CH_2-CH_2-CH_2-CH_2-NH-$ moiety Signals at δ 0 97 (t, 3 H, J = 7 Hz), 2 02 (m, 2 H), 5 4 (m, 1 H), 5 3 (m, 1 H), 2 89 (t, 2 H, J = 7 Hz), 5 4 (m, 1 H), 5 99 (t, 1 H, J = 10 7 Hz), and 5 64 (d, 1 H, J = 10.7 Hz) defined a $CH_3-CH_2-CH=CH-CH_2-CH=CH-CH=C$ side chain The remaining signals at δ 2 77 (m, 2 H), and 2 51 (m, 2 H), each of which collapsed to a broad singlet on irradiation of the other, were assigned to the ring methylene protons in an N-alkyl-γ-methylene-γ-lactam This structure was consistent with the ^{13}C NMR spectrum[5] that contained seven methylene carbon signals between δ 20 3 and 31 1 but did not contain the methine carbon signal expected for the cyclopropene structure We assigned a signal at δ 97 8 (d) to the β-carbon of an enamine (C-5) rather than to a cyclopropenyl carbon Other data that support this structure are the lack of optical activity and the UV absorption at 276 nm (ε 10,000)

The stereochemistry of the side chain was tentatively assigned as 4*E*, 6*Z*, 9*Z* The stereochemistry of the two *Z* olefinic bonds was determined by measuring 11 Hz coupling constants between the olefinic proton signals using the decoupling difference method The assignment of a ^{13}C NMR signal at δ 20.3 (t) to carbon 11 supports the *Z* stereochemistry for the adjacent olefinic bond The remaining olefinic bond was assigned the *E* stereochemistry on the basis of the known preference for the *E* geometry in N-alkyl-γ-methylene-γ-lactams [6]

The ^{1}H NMR spectrum[7] of polyandrocarpidine B (3a) was almost identical to that of polyandrocarpidine A (2a) except in the olefinic region A signal at δ 6 03 (dd, 1 H, J = 15, 11 Hz, C-6) was coupled to signals at 5 42 (d, 1 H, J = 11 Hz) and 5 55 (dt, 1 H, J = 15, 7, 7 Hz), the 15 Hz coupling constant requires the *E* geometry for the $^{6}\Delta$ olefinic bond In the ^{13}C NMR spectrum[7] of polyandrocarpidine B (3a) the C-5 signal was at δ 102 3 (d),[8] 4 5 ppm downfield from the corresponding signal in polyandrocarpidine A (2a) as predicted for a change from *Z* to *E* geometry in the $^{6}\Delta$ olefinic bond

The presence of the N-alkyl-γ-methylene-γ-lactam moiety was confirmed by ozonolysis of a mixture of polyandrocarpidines A-D to obtain the N-alkyl succinimides 5a,b, that were converted into the corresponding dimethylpyrimidyl derivatives 6a,b The infrared bands at 1770 (weak) and 1700 (strong) cm^{-1} were assigned to the succinimide carbonyls The ^{1}H NMR spectrum contained a four proton singlet at δ 2 70 that could only be assigned to the ring protons on an N-alkyl succinimide The major dimethylpyrimidyl derivative 6a was identical in all respects to a sample synthesized from succinic acid and the dimethylpyrimidyl derivative of cadaverine 7, prepared by the method of Cheng and Rinehart [2]

Our data for the minor polyandrocarpidines C (2b) and D (3b) is in agreement with the proposal by Cheng and Rinehart that these molecules have one less methylene group in the N-alkyl-chain than do polyandrocarpidines A (2a) and B (3a) These data, additional data supporting the revised structures for the polyandrocarpidines and details of the synthetic procedures will be presented elsewhere



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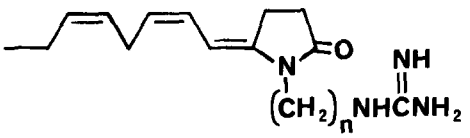

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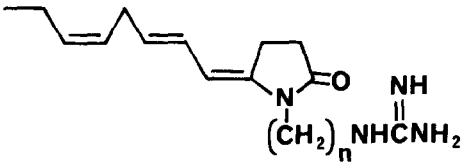

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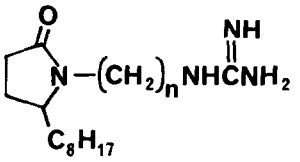

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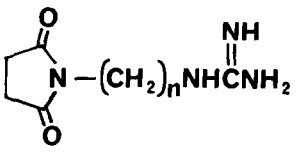

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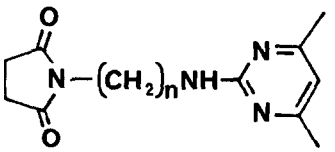

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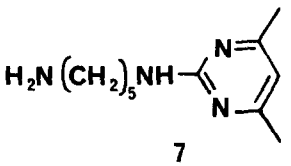

7

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References and Notes

1. R J Andersen and D J. Faulkner, Proc. Food-Drugs Sea, 1972, 111 (1973)
2 M.T Cheng and L K Rinehart, Jr , J. Amer Chem Soc 100, 7409 (1978).
3 The tunicate was collected near La Paz, Bahia San Carlos and Bahia de los Angeles, Mexico by hand using SCUBA (-5 to -15 m)
4 The tetrabutylammonium bisulfate partially obscured some aliphatic signals but comparison of these spectra with spectra of mixed polyandrocarpidines allowed all signals to be located The important olefinic signals were not obscured or shifted by the contaminant
5. Polyandrocarpidine A (2a) ^{1}H NMR ($CDCl_3$) see text, ^{13}C NMR ($CDCl_3$) δ 176.1 (s), 157.2 (s), 141.5 (s), 131 8 (d), 126.5 (d), 126 3 (d), 122 6 (d), 97.8 (d), 41.3 (t), 39 3 (t), 28 6 (t), 27 9 (t), 25 8 (t), 25.6 (t), 23 5 (t), 21.4 (t), 20.3 (t), 14 0 (q)
6 W S Ang and B Halton, Aust J Chem, 24, 851 (1971)
7. Polyandrocarpidine B (3a) ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3 H, J = 7 Hz), 1 35 (m, 2 H), 1 65 (m, 4 H), 2 02 (m, 2 H), 2.51 (m, 2 H), 2 75 (m, 2 H), 2.84 (t, 2 H, J = 7 Hz), 3.04 (q, 2 H, J = 7 Hz), 3 45 (t, 2 H, J = 7 Hz), 5 35 (m, 1 H), 5 42 (d, 1 H, J = 11 Hz), 5 45 (m, 1 H), 5 55 (dt, 1 H, J = 15, 7, 7 Hz), 6 03 (dd, 1 H, J = 15, 11 Hz), 7 07 (bs, NH), 7.73 (bs, 2x NH), ^{13}C NMR ($CDCl_3$) δ 175.9 (s), 157 2 (s), 139 5 (s), 132 2 (d), 128 9 (d), 126 0 (d), 124.3 (d), 102 3 (d), 41.3 (t), 39 3 (t), 30 3 (t), 28.6 (t), 27 9 (t), 25 9 (t), 23 5 (t), 21 4 (t), 20 2 (t), 14 0 (q)
8. This is probably the signal incorrectly assigned to a cyclopropene carbon

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