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CARBON-13 NMR ANALYSIS OF CYCLIC PEPTIDE ALKALOIDS*

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Key Word Index—^{13}C NMR; cyclic peptide alkaloids; discarine-A; discarine-B; lasiodine-A; lasiodine-B; pandamine; pandaminine; hymenocardine.

Abstract—The chemical shifts of the carbons of the peptide alkaloids discarine-A, discarine-B, lasiodine-A, lasiodine-B, pandamine, pandaminine and hymenocardine have been assigned.

The cyclopeptide alkaloids are an expanding group of polyamide plant bases composed of amino acid residues in common and highly modified forms [1]. Compounds sharing the basic skeleton **1**, the *p*-aryloxy macrocyclic peptides, have been the subject of ^{1}H NMR, [1–5] MS [1] and X-ray [6] investigation. This communication presents the ^{13}C NMR analysis of representative substances of this type: discarine-B (**2**), discarine-A (**3**), lasiodine-B (**4**), pandamine (**5**), pandaminine (**6**), hymenocardine (**7**) and of a ring-opened derivative, lasiodine-A (**8**) [7]**.

The 14-membered heterocycle of the substances **2**–**7** imposes conformational constraints on its constituent amino acid residues. These are reflected by disparities from the chemical shifts common to the amino acid residues in a non-terminal position of an acyclic polypeptide. This phenomenon and the occurrence of altered amino acid units necessitated the ^{13}C NMR spectral inspection of some synthetic models.

The carbon shifts of the models Me *N*-acetyltryptophanate (**9**), *N*,*N*,*N*′-triMeisoleucinamide (**10**) and Me *N*,*O*-diacetyl-*β*-hydroxyleucinate (**11**), could be assigned completely by the use of reported chemical shift data of amino acids [8], carbon–hydrogen coupling multiplicity data and established chemical shift parameters [9].

The tryptophan and *N*,*N*′-diMeisoleucine side chains of discarine-B (**2**) possess carbon chemical shifts equivalent to those of models **9** and **10**, respectively. This result was expected, since the side chains are not subject to conformational constraints significantly different from those imposed on the residues of linear polypeptides.

The *β*-aryloxyleucine side chain residue, partly incorporated into the macrocycle, reveals extreme non-equivalence of its *δ*-Me resonances ($\Delta\delta = 5.7$ ppm) in contrast to the (accidentally) degenerate Me resonances of model **11**.

The α-carbon resonances of the tryptophan, *β*-aryloxyleucine and isoleucine residues of **2** are differentiated by comparison with discarine-A (**3**), which differs from discarine-B (**2**) by an interchange of the isoleucine and tryptophan side chains. The α-carbon resonances of each of these residues undergo about a 15 ppm change, reflecting the shift perturbation difference of the *N*-acyl vs *N*,*N*-diMe groups. The α-carbon resonance of the *β*-aryloxyleucine unit remains invariant in the two alkaloids.

The 14-membered macrocycle **1** adopts, in the solid state [6], a conformation in which the normally planar styrene unit of the decarboxydehydrotyrosine residue has its double bond seriously out of the plane of the benzene ring. In solution the restricted motion of the aromatic ring places the 4 aromatic methines in unique, dissimilar environments. Thus, in discarine-B the *ca* 120 ppm signal pair and the *ca* 130 ppm pair can be associated with the centers *ortho* and *meta* to the oxygen function, respectively, but a distinction between the signals in each pair has not been made. The olefinic signals can be differentiated from their aromatic neighbours by the differences in residual, one-bond coupling in the single-frequency off-resonance decoupled spectra [10], but have not been designated individually.

Lasiodine-B (**4**), like the discarines, possesses the macrocycle **1** and exhibits the aromatic methine non-equivalence, which characterizes this unit. The internal proline residue in the side chain exhibits two sets of resonances at ambient temperature arising from the *trans*- and *cis*-isomers of this residue, as noted previously for a large number of synthetic and natural proline-containing peptides [11]. In chloroform solution, lasiodine-B exists as a *ca* 2:1 isomer mixture, the *trans*-isomer, depicted in **4**, predominating [12].

Among the alkaloids **2**, **3** and **4**, one carbonyl signal appears shifted slightly to higher field than the usual amide resonance of polypeptides and is attributed to the

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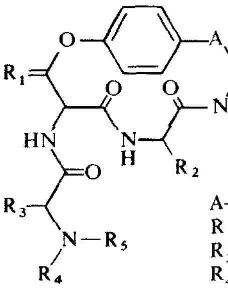
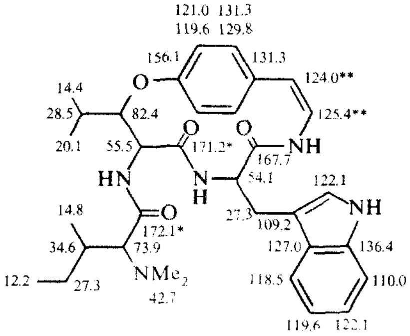
** Long after completion of this study there appeared a ^{13}C NMR analysis of frangulanine [7].



1.

A–B = CH═CH, CHOHCH$_2$, COCH$_2$
R$_1$ = *i*-Pr, H or Me$_2$
R$_2$, R$_3$ = alkyl, arylmethyl
R$_4$, R$_5$ = H, Me, aminoacyl



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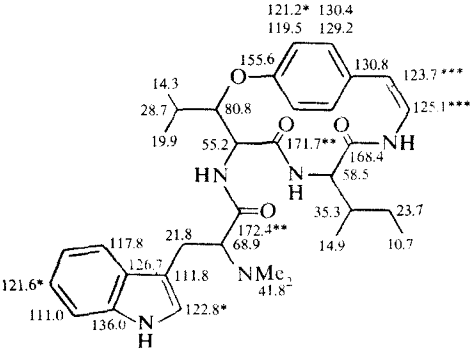

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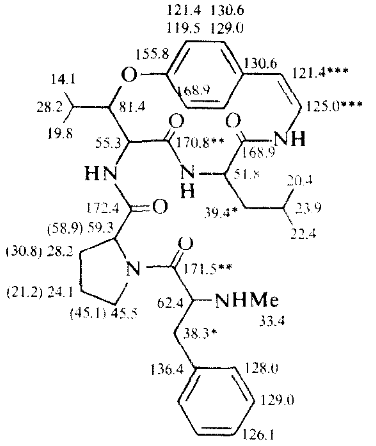

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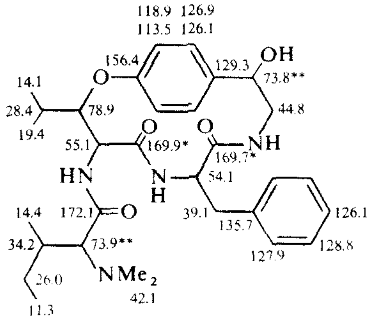

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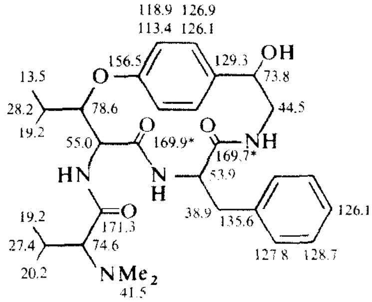

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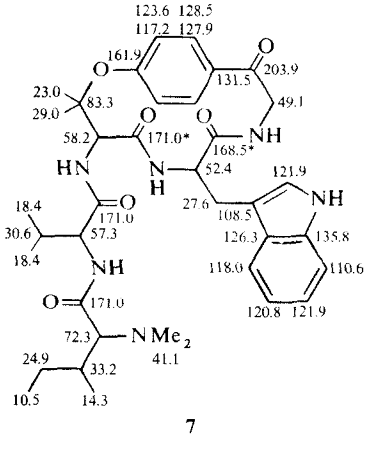

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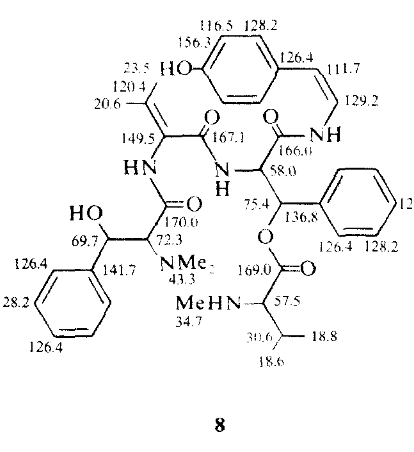

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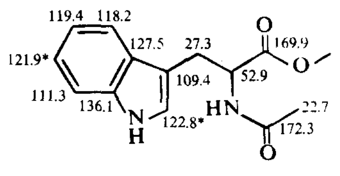

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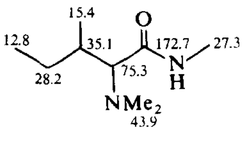

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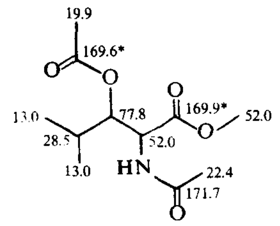

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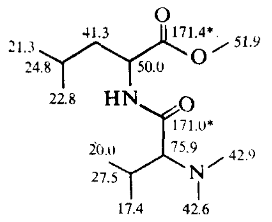

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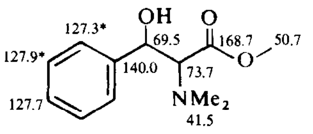

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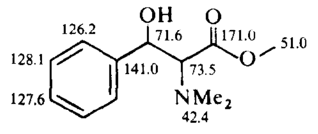

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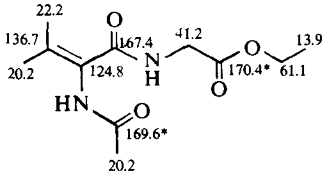

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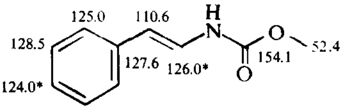

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carbonyl function of the styrylamide moiety. The remaining amide resonances are too close to be assigned with confidence.

Pandamine (**5**) and pandaminine (**6**) are closely related tetrapeptide alkaloids possessing a macrocycle in which the styrene double bond has been hydrated. This perturbation markedly increases the shift non-equivalence of the aromatic methine resonances *ortho* to the oxygen function ($\Delta\delta = 5.4$ ppm) and decreases that of the *meta*-methines ($\Delta\delta = 0.8$ ppm) with respect to the discarine-like alkaloids. The secondary Me resonances of the β-aryloxyleucine residue are insensitive to the hydration. The model Me *N*,*N*-diMevalylleucinate **12** supports the resonance allocation of the valine unit of **6** and provides a reference shift for the leucine Me resonances. Thus, the β-aryloxy substituent in the two alkaloids shields both Me resonances, but does so in a highly discriminating fashion, 8.7 and 1.9 ppm or 7.2 and 3.4 ppm (depending on the assignment), indicative of a strong conformational preference of the side chain.

The macrocycle of hymenocardine (**7**) has a carbonyl group α to the aromatic ring of the decarboxytyrosine residue. In contrast to the *ca* 156 ppm resonance of the oxygenated aromatic carbon in compounds **2**–**6**, this resonance is deshielded 6 ppm in **7**, indicative of substantial resonance interaction between the carbonyl group and the aromatic ring. The conjugation is reflected to a smaller extent at the carbonyl group (203.9 ppm) which is significantly downfield of the ketone resonance in the model *para*-methoxypropiophenone, 197.5 ppm [13]. The deshielding of the carbonyl group of **7** may be attributed to intermolecular hydrogen bonding of the keto function [14, 15] with methanol, a co-solvent with chloroform used to increase the solubility of the alkaloid for the ^{13}C NMR analysis. The chemical shift non-equivalence of the *ortho*- and *meta*-methine resonance pairs attests to the conformationally restricted assymetric environment of the aromatic ring.

The resonances of the tryptophan and isoleucine residues of **7** are identified by their chemical shift similarity with the same residues in **2** and **5**, respectively. The valine residue displays shifts typical for this amino acid located in a non-terminal position of a linear peptide [16].

Lasiodine-A (**8**) is a ring-opened relative of the *para*-aryloxy macrocyclic peptide alkaloids. The two differently substituted β-phenylserine residues and the α,β-unsaturated amide moiety of this substance can be analysed with the aid of the models Me *N*,*N*-diMe-β-phenylserinate in *threo* (**13**) and *erythro* (**14**) forms as well as dipeptide **15**. The aliphatic methine resonances of **13** and **14** are differentiated by qualitative cross correlation of their ^{1}H and ^{13}C NMR spectra [10]. Thus the low-field, *ca* 4.7 ppm benzylic hydrogen resonance of **13** and **14**, results in a larger residual, one-bond ^{13}C–^{1}H splitting in their single-frequency off-resonance decoupled carbon spectra (decoupling frequency at *ca* 0 ppm) for the high-field methine carbon in each pair. In similar fashion, the olefinic Me resonances of model **15** are assigned by specific decoupling of the unambiguous proton resonances.

The low-field signal at 75.4 ppm, a resonance of one of the two oxymethines of lasiodine-A (**8**), is associated with the esterified phenylserine moiety in view of the nominal *ca* 70 ppm shift of this carbon type in models **13** and **14** and the known chemical shift perturbation accompanying acylation [17]. The remaining signal, 69.7 ppm, appears at the general field position characteristic of the oxymethine of *N*,*N*-diMe-β-phenylserine **13** and **14***.

The 2 ppm shift difference of the olefinic methyl resonances of **15** is similar to that in the isopropylidene unit of lasiodine-A (**8**). The *ca* 1 ppm shift deviation of the Me group *cis* to the amide carbonyl function between **15** and **8** may reflect differences in internal hydrogen bonding preferences between the two substances.

The aromatic ring of the decarboxydehydrotyrosine residue of **8**, capable of fast rotation on the NMR time scale, yields easily identifiable shifts on comparison with model **16**. Whereas the olefinic methine resonances of **8** are not diagnostic for *cis*- vs *trans*-isomers of the compound, the *cis* configuration, depicted in formula **8** has been established from the vicinal coupling constant (9.5 Hz) of the olefinic protons [1].

EXPERIMENTAL

The ^{13}C NMR spectra of the alkaloids were recorded on a Varian DP-60 NMR spectrometer operating at 14.1 kG in the Fourier transform mode. $CHCl_3$–MeOH solns (*ca* 2:1 v/v) of the substrates (0.05–0.5 M) were spun in 13 mm OD tubes at ambient temp. Chemical shifts are expressed on the TMS scale according to the following equation $\delta^{TMS} = \delta^{CHCl_3} + 77.2$ ppm. Models **9**–**15** were recorded on the above instrument or one operating at 23.5 kG. The $CHCl_3$ or $CDCl_3$ solvent signal provided a secondary reference: $\delta^{TMS} = \delta^{CHCl_3} + 77.2$ ppm $= \delta^{CDCl_3} + 76.9$ ppm. The asterisks on the formulae indicate possible signal reversal. The δ values in parentheses on formula **4** refer to the proline *cis*-amide.

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* Whereas the data, obtained prior to 1972 on an instrument of low sensitivity (see Experimental), were insufficient to determine the stereochemistry of the *N*,*N*-dimethyl-β-phenylserine moiety, a careful concentration and solvent dependence shift study would nowadays yield such information.

REFERENCES

1. Tschesche, R. and Kaussman, E. U. (1975) in *The Alkaloids* (Manske, R. H. F., ed.) p. 165, Vol. XV. Academic Press, New York; Tschesche, R. (1976) *Heterocycles* **4**, 197.
2. Marchand, J., Païs, M. and Jarreau, F.-X. (1971) *Bull. Soc. Chim. Fr.* 3742.
3. Sierra, M. G., Mascaretti, O. A., Diaz, F. J., Ruveda, E. A., Chang, C.-J., Hagaman, E. W. and Wenkert, E. (1972) *J. Chem. Soc. Chem. Commun.* 915.
4. Chang, C.-J., Hagaman, E. W., Wenkert, E., Sierra, M. G., Mascaretti, O. A., Merkuza, V. M. and Ruveda, E. A. (1974) *Phytochemistry* **13**, 1273.
5. Haslinger, H. (1978) *Monatsh. Chem.* **109**, 523
6. Takai, M., Kawai, K.-I., Ogihara, Y., Iitaka, Y. and Shibata, S. (1974) *J. Chem. Soc. Chem. Commun.* 653; *idem* (1975) *Chem. Pharm. Bull. Jpn* **23**, 2556; Kirfel, A. and Will, G. (1975) *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, **142**, 368.
7. Haslinger, H. (1978) *Tetrahedron* **34**, 685.
8. Horsley, W., Sternlicht, H. and Cohen, J. S. (1970) *J. Am. Chem. Soc.* **92**, 680.
9. Breitmaier, E. and Voelter, W. (1974) *^{13}C NMR Spectroscopy.* Verlag Chemie GmbH, Weinheim/Bergstr.
10. Hagaman, E. W. (1976) *Org. Magn. Reson.* **8**, 389.
11. Deber, C. M., Fossel, E. T. and Blout, E. R. (1974) *J. Am. Chem. Soc.* **96**, 4015 and refs. cited therein.
12. Cf. Thomas, W. A. and Williams, M. K. (1972) *J. Chem. Soc. Chem. Commun.* 994.
13. Dhami, K. S. and Stothers, J. B. (1965) *Can. J. Chem.* **43**, 498.
14. Maciel, G. E. and Ruben, G. C. (1963) *J. Am. Chem. Soc.* **85**, 3903.
15. Maciel, G. E. and Natterstad, J. J. (1965) *J. Chem. Phys.* **42**, 2752.
16. Grathwohl, C. and Wuthrich, K. (1974) *J. Magn. Reson.* **13**, 217.
17. Stothers, J. B. (1972) *Carbon-13 NMR Spectroscopy.* Academic Press, New York.