Phytochemistry, 1974, Vol. 13, pp. 1273 to 1279. Pergamon Press. Printed in England.

PMR SPECTRAL ANALYSIS OF SOME PEPTIDE ALKALOIDS

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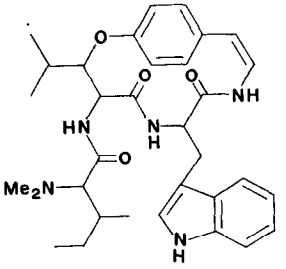
(*Received* 2 *August* 1973)

Key Word Index—Peptide alkaloids; PMR spectral analysis; solvent shifts; discarine B; frangulanine.

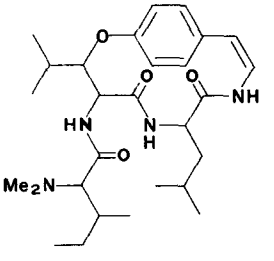
Abstract—220 MHz PMR spectra of the peptide alkaloids discarine B and frangulanine have been recorded and the signals assigned. Studies of solvent and temperature dependence and of deuterium exchange have given data on the conformation of discarine B in solution.

INTRODUCTION

DURING the last decade more than thirty pandamine-like peptide alkaloids have been isolated and largely due to the efforts of the Goutarel and Tschesche groups their structures determined.[1,2] While PMR spectra of the alkaloids have been recorded routinely in this connection, only three efforts have been made to utilize the data for stereochemical diagnosis.[1,3,4] As a consequence it was decided to carry out an exhaustive PMR spectral analysis on some members of this alkaloid type at high resolution, in order to extract as much configurational and conformational detail as possible. The following discussion involves an analysis of the 220 MHz PMR spectra of $CDCl_3$ and d_6-DMSO solutions of discarine B (**1**) and of a d_6-DMSO solution of frangulanine (**2**).[5]



(1) Discarine B



(2) Frangulanine

[1] WARNHOFF, E. (1970) *Fortschr. Chem. Org. Naturstoffe* **28**, 163.
[2] PAÏS M. and JARREAU F. -X. (1971) in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins* (WEINSTEIN, B., ed.), p. 127, Marcel Dekker, New York.
[3] MARCHAND, J., PAÏS, M. and JARREAU, F. -X. (1971) *Bull. Soc. Chim. Fr.* 3742; MARCHAND, J., ROCCHICCIOLI, F., PAÏS, M. and JARREAU, F. -X. (1972) *Bull. Soc. Chim. Fr.* 4699.
[4] GONZÁLEZ SIERRA, M., MASCARETTI, O. A., DIAZ, F. J., RÚVEDA, E. A., CHANG, C.-J., HAGAMAN, E. W. and WENKERT, E. (1972) *J. Chem. Soc. Chem. Commun.* 915.
[5] MASCARETTI, O. A., MERKUZA, V. M., FERRARO, G. E., RÚVEDA, E. A., CHANG, C.-J. and WENKERT, E. (1972) *Phytochemistry* **11**, 1133.

RESULTS AND DISCUSSION

Table 1 portrays the chemical shifts and coupling characteristics of the two alkaloids. Specific signal assignment for the hydrogens of the four amino acid units of discarine B (**1**); the decarboxydehydrotyrosine (*dedetyr*) (**3**), β-hydroxyleucine (*hyleu*) (**4**), tryptophan (*try*)

TABLE 1. CHEMICAL SHIFTS AND COUPLING CONSTANTS*

Amino acid units	**1** ($CDCl_3$, 18°) δ	J	**1** ($CDCl_3$, 48°) δ	J	**1** (d_6-DMSO, 18°) δ	J	**1** (d_6-DMSO, 80°) δ	J	**2** (d_6-DMSO, 80°) δ	J
α-dedetyr	6·48	*m*	6·43	*dd*, 9, 8	6·03	*m*	6·06	*dd*, 8, 7	6·22	*dd*, 8, 7
β-dedetyr	6·27	*d*, 8	6·26	*d*, 8	6·51	*m*	6·42	*d*, 7	6·48	*d*, 8
dedetyr-NH	6·14	*d*, 9	6·11	*d*, 9	7·60	*m*	7·20	*m*	7·20	*m*
α-hyleu	4·43	*dd*, 10, 8	4·42	*dd*, 10, 8	4·41	*dd*, 9, 8	4·41	*dd*, 9, 8	4·40	*dd*, 9, 8
β-hyleu	4·89	*dd*, 8, 2	4·87	*dd*, 8, 2	4·77	*dd*, 8, 2	4·77	*dd*, 8, 2	4·75	*dd*, 8, 2
γ-hyleu	1·50	sept, 7	1·50	sept, 7	1·49	*m*	1·50	*m*	§	§
δ-hyleu	1·21	*d*, 7	1·21	*d*, 7	1·12	*d*, 7	1·14	*d*, 7	1·12	*d*, 7
	0·97	*d*, 7	0·98	*d*, 7	0·94	*d*, 7	0·95	*d*, 7	0·94	*d*, 7
hyleu-NH	7·01	*d*, 10	6·92	*d*, 10	8·25	*d*, 9	7·93	*d*, 9	7·85	*d*, 9
α-try	4·25	*m*	4·25	*td*, 6, 5	4·19	*m*	4·15	*m*		
β-try	3·31	*dd*, 15, 5	3·26	*dd*, 15, 5	2·82	*m*	*ca*2·78	§		
	2·82	*dd*, 15, 6	2·86	*dd*, 15, 6	*ca*2·64	§	*ca*2·78	§		
try-NH	6·01	*d*, 6	5·94	*d*, 6	7·14	*m*	6·78	*d*, 7		
1-try	8·37	*s*	8·15	*s*	10·77	*s*	10·51	*s*		
4-try	7·57	*d*, 8	7·57	*d*, 8	7·31	*d*, 8	7·36	*d*, 8		
7-try	7·30	*d*, 8	7·29	*d*, 8	7·25	*d*, 8	7·27	*d*, 8		
α-ileu(Me_2)	2·44	*d*, 5	2·43	*d*, 5	2·66	*d*, 10	2·65	*d*, 10	2·68	*d*, 10
	*ca*2·01	*m*	*ca*2·00	*m*	*ca*2·13	*m*	*ca*2·11	*m*	§	§
β- and γ-	1·79	*m*	1·78	*m*	1·68	*m*	1·70	*m*	§	§
ileu(Me_2)	*ca*1·26	*m*	*ca*1·26	*m*	§	§	§	§	§	§
δ-ileu(Me_2)	0·94	*t*, 7	0·92	*t*, 7	0·74	*t*, 7	0·76	*t*, 7	0·71	*t*, 7
γ′-ileu(Me_2)	0·87	*d*, 7	0·87	*d*, 7	0·50	*d*, 7	0·57	*d*, 7	0·67†	*d*, 7
ileu(Me_2)-Me	2·02	*s*	2·05	*s*	2·18	*s*	2·19	*s*	2·22	§
α-leu‡									3·90	*dt*, 8, 7
									0·82†	*d*, 7
δ-leu									0·72†	*d*, 7
leu-NH									*ca*6·95	§

* δ values in ppm downfield from TMS; *J* values in Hz.
† Interchangeable shifts.
‡ β-leu and γ-leu hydrogens not discernible because of overlapping signals.
§ Unrecognizable values because of overlapping signals.

(**5**) and *N*,*N*-dimethylisoleucine [*ileu*(Me_2)] (**6**) units; from the PMR spectrum of a $CDCl_3$ solution is based on shift position, multiplicity and specific decoupling (Fig. 1). Overlapping of the signals of the aromatic hydrogens of the decarboxydehydrotyrosine unit with those of tryptophan's 2-H, 5-H and 6-H in the 6·7–7·1 ppm region preclude their exact analysis. Shift assignment of the hydrogens of **1** in d_6-DMSO is based on spectra in solvent mixtures (Table 2). A study of the deuterium exchange of the amide hydrogens in a 14% d_6-DMSO solution (*vide infra*) confirms the designation of the δ values in the α-hydrogens. The PMR spectrum of discarine B (**1**) in the sulfoxide solvent serves as a model for the shift assignment of the hydrogens of frangulanine (**2**). The shifts of the secondary methyl groups of the *ileu*(Me_2) and *leu* moieties unfortunately cannot be differentiated.

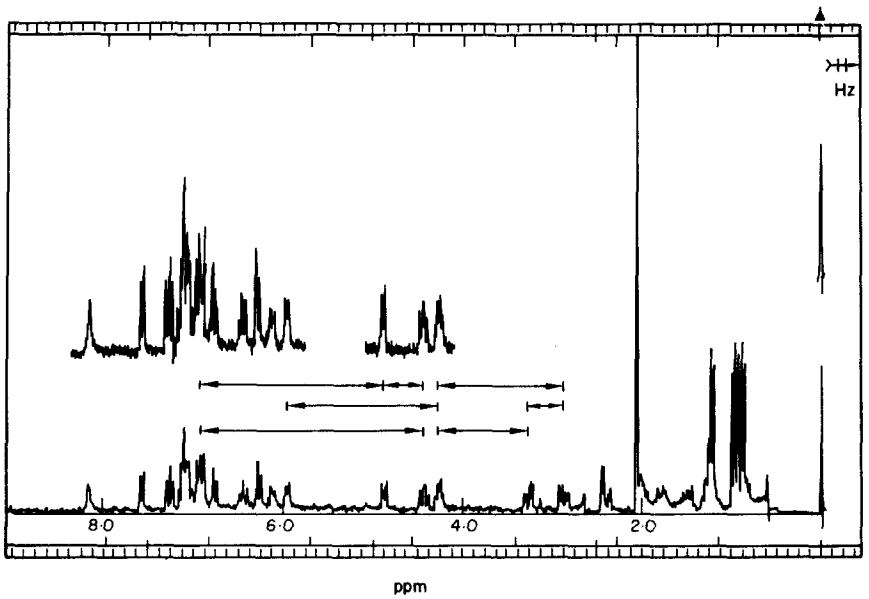
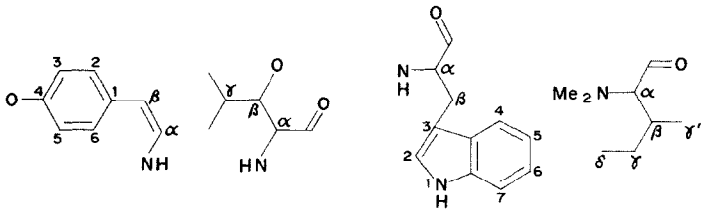


FIG. 1. 220 MHz PMR SPECTRUM OF DISCARINE B IN $CDCl_3$.
(The two-pointed arrows refer to the signals of hydrogen pairs which were decoupled.)

TABLE 2. SHIFT DEPENDENCE OF DISCARINE B ON SOLVENTS

Amino acid units	$CDCl_3$		4% d_6-DMSO		8% d_6-DMSO		11% d_6-DMSO		14% d_6-DMSO	
	δ	J	δ	J	δ	J	δ	J	δ	J
α-dedetyr	6·48	*m*	*ca*6·41	*m*	*ca*6·36	*	*ca*6·28	*m*	*ca*6·25	*m*
β-dedetyr	6·27	*d*, 8	*ca*6·30	*	*ca*6·34	*	6·38	*d*, 8	6·41	*d*, 8
dedetyr-NH	6·14	*d*, 9	*ca*6·36	*	6·57	*m*	6·74	*m*	*ca*6·84	*m*
α-hyleu	4·43	*dd*, 10, 8	4·43	*dd*, 10, 8	4·42	*dd*, 10, 8	4·41	*dd*, 10, 8	4·41	*dd*, 10, 8
β-hyleu	4·89	*dd*, 8, 2	4·88	*dd*, 8, 2	4·85	*dd*, 8, 2	4·84	*dd*, 8, 2	4·84	*dd*, 8, 2
γ-hyleu	1·50	sept†, 7	1·52	sept†, 7	1·53	sept†, 7	1·54	sept†, 7	1·55	*m*
	1.21	*d*, 7	1·20	*d*, 7	1·20	*d*, 7	1·19	*d*, 7	1·18	*d*, 7
δ-hyleu	0·97	*d*, 7	0·97	*d*, 7	0·97	*d*, 7	0·96	*d*, 7	0·96	*d*, 7
hyleu-NH	7·01	*d*, 10	7·34	*d*, 10	7·61	*d*, 10	7·76	*d*, 10	7·89	*d*, 10
α-try	4·25	*m*	4·22	*m*	4·18	*m*	4·18	*m*	4·18	*m*
	3·31	*dd*, 15, 5	3·24	*dd*, 15, 5	3·18	*dd*, 15, 5	3·13	*dd*, 15, 5	3·09	*dd*, 15, 5
β-try	2·82	*dd*, 15, 6	2·85	*dd*, 15, 6	2.84	*dd*, 15, 6	2.84	*dd*, 15, 6	2·84	*dd*, 15, 6
try-NH	6·01	*d*, 6	6·16	*d*, 6	6·27	*d*, 6	6·35	*d*, 6	*ca*6·39	*
1-try	8·37	*s*	9·39	*s*	9·92	*s*	10·15	*s*	10·29	*s*
4-try	7·57	*d*, 8	7·53	*d*, 8	7·49	*d*, 8	7·47	*d*, 8	7·45	*d*, 8
7-try	7·30	*d*, 8	7·32	*d*, 8	7·32	*d*, 8	7·31	*d*, 8	7·30	*d*, 8
α-ileu(Me_2)	2·44	*d*, 5	2·51	*d*, 6	2·56	*d*, 7	2·58	*d*, 8	2·59	*d*, 9
	*ca*2·01	*m*	*ca*2·09	*	*ca*2·11	*	*ca*2·12	*	*ca*2·12	*
β- and γ-	1·79	*m*	1·81	*m*	1·81	*m*	1·81	*m*	1·81	*m*
ileu(Me_2)	*ca*1·26	*m*	*ca*1·24	*	*ca*1·24	*	*ca*1·24	*	*ca*1·24	*
δ-ileu(Me_2)	0·94	*t*, 7	0·91	*t*, 7	0·89	*t*, 7	0·88	*t*, 7	0·86	*t*, 7
γ′-ileu(Me_2)	0·87	*d*, 7	0·83	*d*, 7	0·80	*d*, 7	0·78	*d*, 7	0·76	*d*, 7
ileu(Me_2)-Me	2·02	*s*	2·10	*s*	2·14	*s*	2·16	*s*	2·17	*s*

* Unrecognizable values because of overlapping signals.
† Broad signal.



(3) (4) (5) (6)

The NH groups of discarine B (**1**) show both solvent and temperature dependence. In consonance with previous observations[6] the sulfoxide solvent deshields strongly the NH functions. The tryptophan amide unit shows the weakest-effect and the indole NH the strongest. This fact suggests that the amide moiety is most protected, possibly by hydrogen bonding with the carbonyl group of the isoleucine unit. Little information is gained from an inspection of the temperature coefficients of the NH groups of **1**.[7] However, more interpretable data are obtained from a qualitative study of the rate of exchange of the *N*-bonded hydrogens with deuterons from *ca* 1% D_2O. While the exchange is instantaneous for the NH groups of the subunits **3** and **4** at 18° in 14% of d_6-DMSO in $CDCl_3$ solution, both NH functions of **5** remain unfazed. At 45° after 25 min the amide and indole NH groups of **5** undergo 17 and 86% exchange, respectively, whereas on addition of *ca* 1% of trifluoroacetic acid total exchange of the amide NH occurs at 45° after 45 min. In d_6-DMSO at 18° instantaneous exchange takes place in the subunits **3** and **4** to the extent of 30 and 75%, respectively, while the amide of **5** remains unchanged. At 40° after 30 min the amides of **3** and **4** are exchanged 80 and 90%, respectively. In the presence of *ca* 1% trifluoroacetic acid at 18° the amide hydrogen of **5** undergoes instantaneous exchange of 38%. These results show once again that in contrast to the other amide NH group the hydrogen of the tryptophan amide is strongly protected from the basic solvent. While this could be a consequence of steric hindrance from the vicinal skatyl and isoleucine units, it is more likely due to an intramolecular hydrogen bond, as the difference of effect by trifluoroacetic acid on the various amide linkages indicates. The additive deshields the NH groups of **3** and **4** 0·23 and 0·45 ppm, respectively, while leaving the amide of tryptophan nearly unchanged ($\Delta\delta = -0{\cdot}05$ ppm).[9] The almost complete immunity of the coupling constants of hydrogens on vicinal carbon sites in the 14-membered ring of discarine B (**1**) to solvent and temperature changes (Table 1) suggests minimal conformational change.[10]

The change of solvent from $CDCl_3$ to d_6-DMSO and the addition of trifluoroacetic acid have an effect not only on the NH groups, but also on other protonated centers. The acid exerts a strong deshielding effect on the α-H and the *N*-methyl groups of **6**,

[6] TORCHIA, D. A., DI CORATO, A., WONG, S. C. K., DEBER, C. M. and BLOUT, E. R. (1972) *J. Am. Chem. Soc.* **94**, 609.

[7] In $CDCl_3$ they are 1, 3, 2 and 7 × 10^{-3} ppm/deg and in d_6-DMSO 11, 8, 9 and 6 × 10^{-3} ppm/deg for the amides of **3**, **4**, **5** and the indole NH, respectively. This unfortunately indicates that in $CDCl_3$ all NH groups except the indole 1-H are protected and in d_6-DMSO exposed to the solvent.[8]

[8] PEASE, L. G., DEBER, C. M. and BLOUT, E. R. (1973) *J. Am. Chem. Soc.* **95**, 258.

[9] In contrast to the above results Warnhoff[1] has claimed that the amide group of **3** in the peptide alkaloids undergoes slowest hydrogen exchange. Since the chemical shift of this NH group was not listed, no explanation for this discrepancy can be given.

[10] BYSTROV, V. F., IVANOV, V. T., PORTNOVA, S. L., BALASHOVA, T. A. and OVCHINNIKOV, YU. A. (1973), *Tetrahedron* **29**, 873; and references therein.

1·21 and 0·66 ppm in $CDCl_3$ and 1·02 and 0·56 ppm in d_6-DMSO, respectively, in view of the protonation of the dimethylamino function.[1,11] The dramatic shielding of the olefinic α-H of the decarboxydehydrotyrosine unit and of 4-H and one of the methylene hydrogens of the tryptophan moiety with increasing concentration of d_6-DMSO in $CDCl_3$ indicates strong solute-solvent interaction between one face of the peptide frame with the polar solvent. This may be a consequence of dipole–dipole involvement of mainly the decarboxydehydrotyrosine peptide function with the solvent or even stronger interaction by way of hydrogen bonding of the solvent with the exposed NH of the decarboxydehydrotyrosine amide. The striking solvent effect on only one hydrogen of the tryptophan methylene group reflects a preferred rotamer population of the skatyl unit, a fact confirmed by the high-field position of the γ′-hydrogens of the isoleucine moiety. The shielding of this methyl group, increasing with higher concentration of d_6-DMSO, indicates the methyl function to be positioned over the π-electron cloud of the indole ring. This methyl shielding is maintained in dihydrodiscarine B [γ′-ileu(Me_2) 0·60 ppm (*d*, *J* 7 Hz) in d_6-DMSO].[12,13]

[11] MA, J. C. N. and WARNHOFF, E. W. (1965) *Can. J. Chem.* **43**, 1849.

[12] In pyridine-d_5 solution the methyl groups of discarine B and its dihydro derivative experience a shift change. **1**: δ-hyleu 1·22, 1·32 ppm (*d* each, *J* 7 Hz); δ-ileu(Me_2) 0·80 (*t*, 7); γ′-ileu(Me_2) 0·95 (*d*, 7). Dihydro-**1**: δ-hyleu 1·22, 1·33 ppm (*d* each, *J* 7 Hz); δ-ileu(Me_2) 0·80 (*t*, 7); γ′-ileu(Me_2) 1·01 (*d*, 7).

[13] The chemical shifts of the methyl groups of discarine A^5 in pyridine-d_5 are: δ-hyleu 1·17, 1·20 ppm (*d* each, *J* 7 Hz); δ-ileu 0·66 (*t*, 7); γ′-ileu 0·83 (*d*, 7). Those of the methyl substituents in dihydrodiscarine A^5 in pyridine-d_5 are: δ-hyleu 1·07, 1·09 ppm (*d* each, *J* 7 Hz); δ-ileu 0·67 (*t*, 7); γ′-ileu 0·86 (*d*, 7).