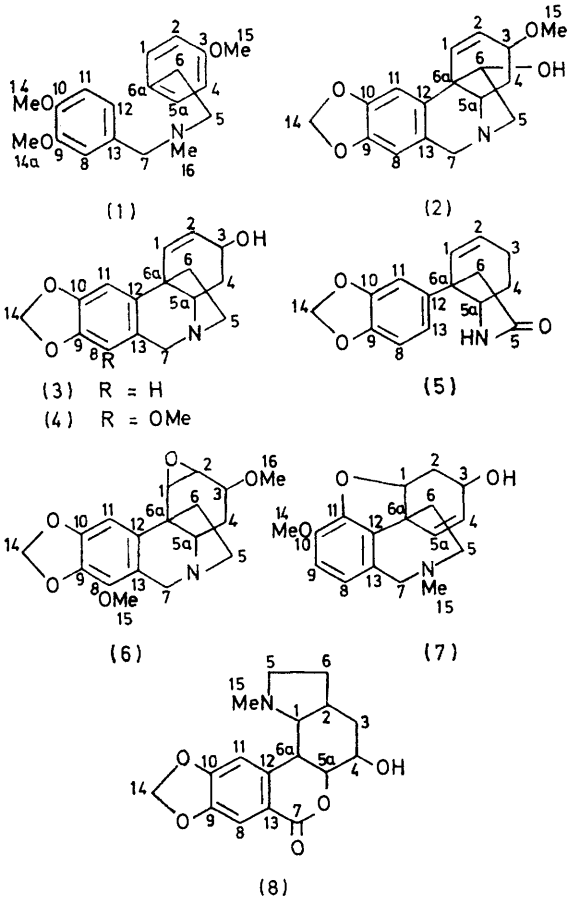
^{13}C Nuclear Magnetic Resonance Spectra of Amaryllidaceae Alkaloids

By **Lucia Zetta** and **Giuseppe Gatti,*** Istituto Chimica Macromolecole, Via A. Corti 12, 20133 Milano, Italy
Claudio Fuganti, Istituto Chimica del Politecnico, Piazza L. da Vinci 32, 20133 Milano, Italy

The ^{13}C n.m.r. spectra of the title compounds are reported with complete assignments, based on peak multiplicity, single frequency proton decoupling, the use of lanthanide shift reagents, and empirical calculations of chemical shifts.

^{13}C NUCLEAR magnetic resonance spectroscopy is being increasingly directed towards the study of relatively large molecules, such as naturally occurring



compounds, the assignment of the peaks in the spectra providing valuable structural information. To date there is only one report on the ^{13}C n.m.r. spectra of the Amaryllidaceae alkaloids [1] and this work was undertaken with the purpose of contributing towards a fuller understanding of the ^{13}C n.m.r. spectra of this class of compounds. We report the results of a study of eight molecules of varying molecular complexity but of similar chemical functionality.

Except for compound (5), an intermediate in the synthesis of crinine (4), all the alkaloids examined are relatively abundant. Four of them, haemanthanine (2), undulatine (6), and the naturally occurring 1 : 1 mixture of crinine (4) and powelline (3) have the crinane skeleton which is derived biosynthetically from norbelladine [compound (1) without the four methyl groups] by phenol coupling and which is biologically degraded to the lactam narciclasine which has already been examined.[2] Galanthamine (7) represents a rather uncommon skeletal type and clivonine (8) is unique in this class of compounds because it is present as an ester component only in Clivia.

The spectrum of (3) and (4) was recorded without separating the two components, and this is a clear example of the power of the ^{13}C technique in analysing mixtures of complex molecules.

EXPERIMENTAL

The ^{1}H and natural abundance ^{13}C spectra were recorded with an HFX-90 Bruker spectrometer at 90 and 22·63 MHz respectively with Me_4Si as internal standard. The samples were 0·5M solutions in $CDCl_3$ with heteronuclear lock. The spectrum of compound (5) was recorded using the Fourier transform technique because of its low solubility and the others by the continuous-wave mode. Representative spectra are shown in Figures 2 and 4 and the chemical shifts are collected in Table 1 and Figure 3.

DISCUSSION

The interpretation of the spectra is based on the ^{13}C chemical shifts, on the multiplicities shown in the off-resonance decoupled spectra, and on the frequency of selective proton decoupling (when the individual proton signals are sufficiently separated). When this was not the case, *i.e.* for belladine (1), the proton spectrum was expanded by adding $Eu(DPM)_3$ to the

[1] W. O. Crain, jun., W. C. Wildman, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1971, **93**, 990.

[2] L. Zetta, G. Gatti, and C. Fuganti, *Tetrahedron Letters*, 1971, 4447.

alkaloid solution.* The effect of lanthanide addition (see Figure 2) is apparently greatest on the 14- and 14a-methoxy-groups and decreases in the series 11-,

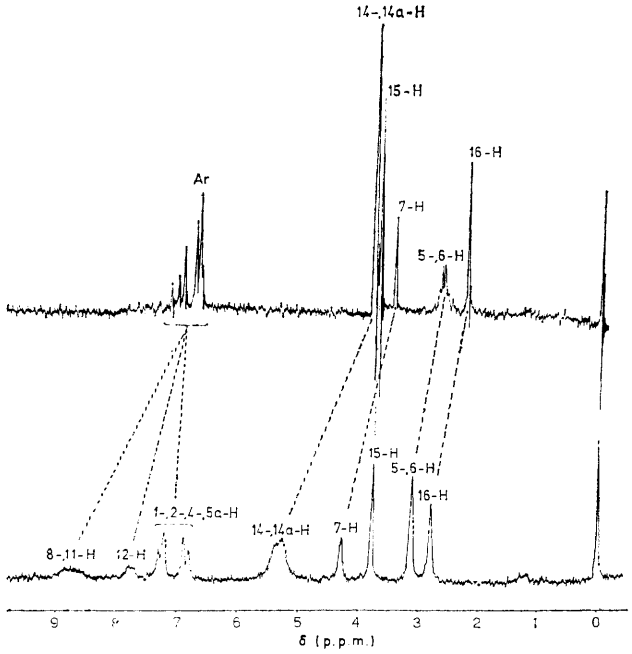


FIGURE 1 ^{1}H N.m.r. spectra of belladine (1): upper trace, in the absence of Eu(DPM)$_3$; lower trace, in the presence of Eu(DPM)$_3$

8-, 12-, and 7-H, the remaining protons being only slightly shifted. On this basis one may deduce that the molecular region involved in complex formation is that of the two oxygen atoms of the 14- and 14a-methoxy-group,

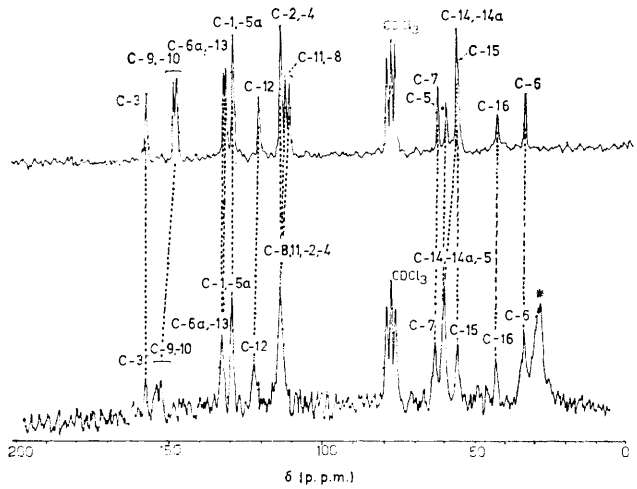


FIGURE 2 Proton-decoupled ^{13}C n.m.r. spectra of belladine (1): upper trace, in the absence of Eu(DPM)$_3$; lower trace, in the presence of Eu(DPM)$_3$

by consideration of the well known relationship between the inverse of the distance between the Eu atom and the shift of the proton due to pseudo-contact interaction.[3] The enhanced separation induced between the signal for 5- and 7-H$_2$ allowed selective decoupling of these two groups and consequently the corresponding assignment in the ^{13}C spectrum.

Moreover, the addition of the lanthanide reagent also facilitated some assignments in the ^{13}C n.m.r. spectrum. Since complexation involved the substituents in only one of the aromatic rings it was possible to distinguish the carbon resonances of this ring as they were considerably shifted in comparison with those of the other ring, as will be described.

The ^{13}C n.m.r. spectra of all the compounds examined were divided in two well defined regions. The lowfield (>δ 90) region contained the carbonyl group signals, if any, the unsaturated carbon signals, both aromatic or olefinic, and the methylenedioxy carbon signal; all the remaining saturated carbon resonances were located in the highfield region. For simplicity the two regions will be discussed separately.

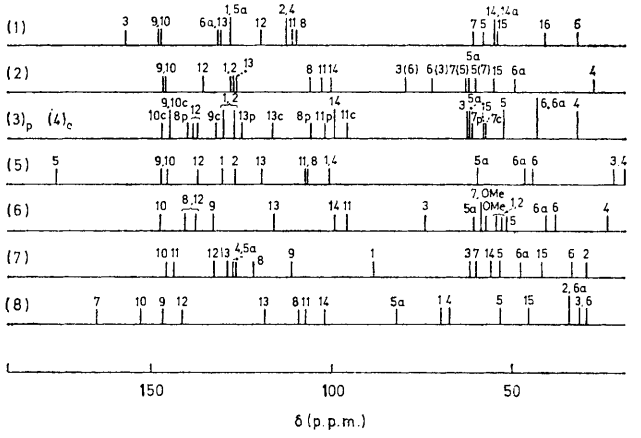


FIGURE 3 Correlation of ^{13}C chemical shifts of Amaryllidaceae alkaloids

Lowfield Resonances.—The signal of the methylenedioxy carbon, present in the majority of the molecules examined, exhibits a characteristic shift at δ 103, the assignment being confirmed by its triplet structure in the off-resonance decoupled spectrum, which is unique in the lowfield region.

The carbonyl peaks may be recognized on the basis that their shift is always > δ 160. The amido carbonyl in narciclasine acetate [2] and the ester carbonyl in clivonine are in comparable environments and accordingly absorb at about the same frequency (as do, for instance, the carbonyl carbons of methyl acetate and dimethylacetamide). The observed upfield shift relative to the amide group in compound (5) and the ester group in narciclasine is apparently due to conjugation with the phenyl ring which has a well known shielding effect.[4]

The signals due to the unsaturated carbon atoms,

* The assignments for both diamagnetic and paramagnetic solutions are based on the chemical shifts and on the intensity pattern.

[3] C. C. Hinckley, *J. Amer. Chem. Soc.*, 1969, **91**, 5160.

[4] E. Lipmaa, T. Pehk, K. Andersson, and C. Rappe, *Org. Magnetic Resonance*, 1970, **2**, 109.

TABLE 1

^{13}C Chemical shifts * and multiplicity

	C-1	C-2	C-3	C-4	C-5	C-6	C-5a	C-6a	C-7
(1)	129·5 (d)	114·2 (d)	158·3 (s)	114·2 (d)	59·8 (t)	33·5 (t)	129·5 (d)	132·9 (s) 132·0 (s)	62·8 (t)
	(129·1)	(114·1)	(157·2)	(114·1)			(129·1)		
(2)	127·2 (d) 128·0 (d)	128·0 (d) 127·2 (d)	80·0 (d) 73·0 (d)	29·5 (t)	61·5 (t) 63·3 (t)	73·0 (d) 80·0 (d)	62·7 (d)	50·0 (s)	63·3 (t) 61·5 (t)
(3)	127·2 (d) 129·5 (d)	129·5 (d) 127·2 (d)	63·5 (d)	33·5 (t)	54·8 (t)	44·7 (t)	62·6 (d) 63·0 (d)	44·7 (s)	62·2 (t)
(4)	127·2 (d) 129·5 (d)	129·5 (d) 127·2 (d)	63·5 (d)	33·5 (t)	54·8 (t)	44·7 (t)	63·0 (d) 62·6 (d)	44·7 (s)	58·6 (t)
(5)	130·8 (d)	127·5 (d)	24·0 (t) 20·5 (t)	20·5 (t) 24·0 (t)	176·5 (s)	45·8 (t)	61·0 (d)	48·0 (s)	
(6)	52·5 (d) 54·0 (d)	54·0 (d) 52·5 (d)	73·0 (d)	24·5 (t)	51·0 (t)	38·0 (t)	60·0 (d)	40·0 (s)	57·5 (t)
(7)	88·1 (d)	30·0 (t)	62·2 (d)	126·0 (d) 126·8 (d)	54·3 (t)	34·0 (t)	126·8 (d) 126·0 (d)	48·2 (s)	60·5 (t)
(8)	70·2 (d)	34·0 (d)	29·5 (t) 32·9 (t)	68·5 (d)	53·9 (t)	32·0 (t) 29·5 (t)	82·8 (d)	34·0 (s)	164·7 (s)
	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16
(1)	111·7 (d) 112·9 (d)	149·0 (s) 148·2 (s)	148·2 (s) 149·0 (s)	112·9 (d) 111·7 (d)	121·6 (d)	132·0 (s)	56·0 (q)	55·7 (q)	42·0 (q)
	(112·2)	(149·9)	(148·4)	(112·2)	(121·1)	(132·6)			
(2)	106·9 (d)	146·5 (s) 147·0 (s)	147·0 (s) 146·5 (s)	103·3 (d)	135·0 (s)	126·9 (s)	101·0 (t)	56·0 (q)	
	(108·2)	(144·8)	(145·7)	(105·6)		(130·1)			
(3)	106·0 (d)	145·0 (s) 145·2 (s)	145·2 (s) 145·0 (s)	102·0 (d)	137·0 (s) 138·0 (s)	124·9 (s)	99·3 (t)		
	(108·2)	(144·8)	(145·7)	(105·6)		(130·1)			
(4)	140·0 (s)	132·5 (s)	147·0 (s)	96·0 (d)	138·0 (s) 137·0 (s)	116·0 (s)	99·3 (t)	59·3 (q)	
	(140·2)	(130·4)	(146·7)	(97·9)		(115·7)			
(5)	107·2 (d) 108·0 (d)	146·5 (s) 147·8 (s)	147·8 (s) 146·5 (s)	108·0 (d) 107·2 (d)	137·8 (s)	120·5 (d)	101·9 (t)		
	(108·2)	(144·8)	(147·2)	(105·6)		(118·6)			
(6)	139·5 (s) 137·0 (s)	131·0 (s)	146·5 (s)	94·0 (d)	137·0 (s) 139·5 (s)	116·0 (s)	98·3 (t)	57·5 (q) 56·0 (q)	56·0 (q) 57·5 (q)
	(140·2)	(130·4)	(146·7)	(97·9)		(115·7)			
(7)	121·6 (d)	110·5 (d)	145·5 (s)	144·0 (s)	132·7 (s)	129·5 (s)	56·0 (q)	42·2 (q)	
	(120·6)	(109·3)	(147·9)	(146·8)		(132·1)			
(8)	109·5 (d)	146·8 (s)	152·5 (s)	107·8 (d)	141·2 (s)	119·0 (s)	102·7 (t)	46·0 (q)	
	(110·8)	(145·3)	(151·5)	(107·0)		(122·3)			

* In p.p.m. from Me_4Si (measured from $CDCl_3$ = δ 76·9). Ambiguous assignments are reported twice; calculated values are in parentheses.

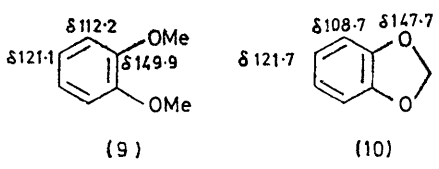
both olefinic and aromatic, were assigned in the following way. The olefinic carbon atoms present in compounds (2)—(5) and (7) were assigned from their typical shift between δ 126 and 130 as already observed for similar compounds.[1] For the aromatic carbons a distinction was made between CH and –C= groups on the basis of the off-resonance multiplicity of the signals, doublets and singlets respectively. The positions of the individual signals were then calculated by adding to the shift observed in model compounds the contribution of the substituent shielding parameters (see Table 2). The model compounds examined were dimethoxybenzene (9) and methylendioxybenzene (10). The shifts of the trisubstituted aromatic ring in compound (1) were calculated by adding to those measured in (9) the contributions of a CH_2NMe_2 group which is quite similar for our purpose to the substituent at C-13. The shifts of the *para*-disubstituted ring were

TABLE 2

^{13}C Substituent n.m.r. shift parameters (p.p.m.)

Substituent	C-1	*ortho*	*meta*	*para*	Ref.
OMe	+31·4	−14·4	+1·0	−7·7	*a*
$(CH_2)_2N$	+10·8	−0·6	−0·2	−2·9	*b,c*
Bu^t	+22·3	−3·1	−0·5	−2·9	*c*
Pr^i	+20·1	−1·9	−0·1	−2·6	*c*
CH_2NMe_2	+11·5			−1·5	*d*
CO_2R	+2·5	+2·2	+0·2	+3·9	*e*

[a] G. C. Levy, G. L. Nelson, and J. D. Cargioli, *Chem. Comm.*, 1971, 506. [b] Values obtained by adding to the parameter for Et the effect of a nitrogen atom (T. D. Brown, Ph.D. Thesis, University of Utah, 1966). [c] H. Hasegawa, M. Imanari, and K. Ishizu, *Bull. Chem. Soc. Japan*, 1972, **45**, 1153. [d] L. Zetta and G. Gatti, *Org. Magnetic Resonance*, 1972, **6**, 585. [e] K. S. Dhami and J. B. Stothers, *Canad. J. Chem.*, 1966, **44**, 2855.



(9) (10)

likewise calculated by adding to the benzene shift for the contributions of OMe and $(CH_2)_2N$ groups.

The agreement reported in Table 1 (calculated values in parentheses) shows that the additivity rule is in this case a good approximation and allows the assignments indicated; obviously signals very close to each other such as C-9 and C-10 or C-6a and C-13 are not unambiguously assigned.

The same procedure was applied to compound (5) by using (10) as a model compound to which the contribution of a t-butyl group was added as an approximation to the substituent at C-12. Here again agreement is satisfactory with the exception of C-12 for which t-butyl contribution is a very poor approximation as will be seen for all the compounds studied. However, C-12 was assigned by elimination since it is the only singlet in the aromatic region besides C-9 and -10.

For the compounds with tetrasubstituted aromatic rings [(2), (3), and (8)] the same model compound (10) can be used, adding substituent contributions at C-12 and -13. The approximations were: (a) for C-12, Bu^i for (2) and (3), Pr^i for (8); and (b) for C-13, CH_2NMe_2 for (2) and (3), CO_2R for (8). The agreement shown in Table 1 is apparently less satisfactory than that obtained for the trisubstituted aromatic rings, but however it reproduces the observed pattern. For the remaining tetrasubstituted aromatic ring in compound (7) the model compound (9) was used with the parameters for the substituents Bu^t and CH_2NMe_2, a good agreement being obtained.

Finally the pentasubstituted ring absorptions in compounds (4) and (6) were calculated by adding to the model (10) the contribution of the three substituents at C-8, -13, and -12 as before. Despite the large number of *ortho*-interactions the additivity rule provides a basis for the assignments of the five singlets for C-8, -9, -10, -12, and -13.

These assignments allow a re-examination of the spectrum of narciclasine acetate.[2] The chemical shift of C-10 and -12 in the majority of the compounds studied suggests that the peaks at δ 153 and 143 are due to C-10 and -12 respectively. Consequently the absorptions for C-16a and -9 may be attributed to the signal in the region between δ 130 and 135.

Highfield Resonances.—The only characteristic group frequency is that of NMe which is present in (1), (7), and (8) and gives rise to an easily recognizable signal in the region δ 42—46,[5] on the basis of the quartet in the off-resonance decoupled spectrum.

In (8), since off-resonance decoupling allows only a rough distinction to be made between the five CH and the three CH_2 groups, extensive use of single-frequency proton decoupling has been made to aid further assignments. Thus irradiation of 4-, of 5a-, and of 1-H in the ^1H spectrum[6] resulted in the selective collapse of doublets at δ 68·5, 82·8, and 70·2 respectively. The remaining two CH groups at C-2 and -6a are, by elimination, assigned to the strong signal (doublet in off-resonance decoupled spectrum) at δ 34·0. Finally irradiation in the highfield region of the ^1H spectrum, *i.e.* of 3-H_2, resulted in coalescence in the ^{13}C spectrum of the two triplets at δ 31, one of which is therefore assigned to C-3. Of the two remaining methylene groups at C-5 and -6, the former is expected to absorb at lower field due to the deshielding effect of the nitrogen atom (as already observed for C-1 relative to C-2). Therefore it is reasonable to assign C-5 to the triplet at δ 53·9, and C-6 to one of the two triplets at δ *ca.* 31.

The aliphatic part of the spectrum of (1) consisted of three CH_2 triplets, of which those due to C-7 and -5 were assigned on the basis of their lowfield shift relative to that of the remaining triplet; single-frequency proton decoupling from 7-H_2 allowed the distinction between C-5 and -7 to be made; the highfield triplet was, by elimination, assigned to C-6.

The assignment in the ^1H spectrum (Figure 2) of 5- and 7-H_2 is based on the fact that the signal for 7-H_2 is a singlet while that for 5-H_2 is part of a deceptively simple AA'BB' spectrum. The absorption at δ 56 is due to the three OMe groups. Addition of $Eu(DPM)_3$ resulted in a considerable shift of the lowfield signal, while the highfield signal remained practically unaltered. Therefore the more intense lowfield component was assigned to C-14 and -14a.

In compound (5) the two highfield triplets were located in the typical region of the α- and β-carbon atoms of cyclohexene[7] so that the signal at δ 45·8 was, by elimination, assigned to C-6.

In compound (2), the triplet at highfield with a shift characteristic of an alicyclic methylene carbon is clearly due to C-4 while the assignment of triplets for C-5 and -7 is uncertain. The two doublets shifted towards lowfield may be assigned to C-3 and -6 because of the strong deshielding effect of the oxygen atom. The remaining doublet at δ 62·7 is therefore due to C-5a.

In the aliphatic region of the spectrum of the mixture of (3) and (4) the signals due to the carbon atoms remote from C-8, which in one case carries the OMe substituent, are coincident in the two molecules, while those of the carbon atoms close to C-8 can be differentiated. Thus the C-4 triplet at δ 33·5 and the C-6a singlet overlapping with the C-6 triplet at δ 44·7 cannot be resolved, even on expansion of the spectrum; their relatively highfield position is in keeping with the cases examined previously. In the expanded spectrum (Figure 4) of the region between δ 30 and 70, the quartet at δ 59·3 is due to the OMe group present only in (4). The more intense doublet at δ 63·5 was attributed to C-3 in both molecules, while the C-5a signals are distinguished by the long range effect of the OMe group. The absorption of the C-5 triplets are almost coincident in the two species (δ 54·8) while the remaining triplets due to

[5] I. Morishima, K. Okada, T. Yonezawa, and K. Goto, *J. Amer. Chem. Soc.*, 1971, **93**, 3922.

[6] P. W. Jeffs, J. F. Hansen, W. Dopke, and M. Bienert, *Tetrahedron*, 1971, **27**, 5065.

[7] D. E. Dorman, N. Jautelat, and J. D. Roberts, *J. Org. Chem.*, 1971, **36**, 2757.

C-7 are widely separated, the highfield component being due to the crinine [shielding effect of OMe, *cf.* the similar situation for undulatine (6)].

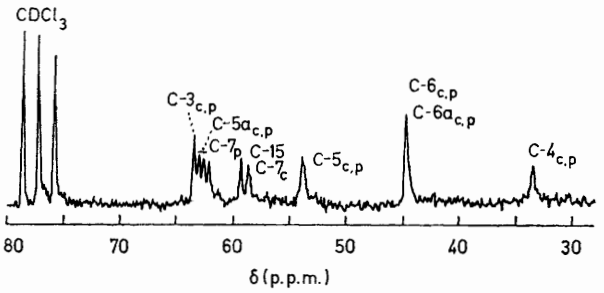


FIGURE 4 Expanded spectrum of the mixture of powelline (3) (p) and crinine (4) (c)

In the spectrum of (7) the two CH doublets were assigned by single-frequency proton decoupling. The corresponding assignments in the ^1H spectrum were obtained by irradiation of the olefinic protons which allows 3- to be distinguished unequivocally from 1-H. There are two kinds of methylene signals present in the ^{13}C spectrum. Those from carbons bonded to nitrogen were assigned to the lowfield triplets at δ 60·5 and 54·3, the former having the typical shift of the benzylic C-7 already observed in the compounds discussed above. Of the remaining highfield triplets the signal of C-6 was assigned by single-frequency decoupling since the corresponding proton absorption is sufficiently separated from that of 2-H_2.

In compound (6) (Figure 3) the triplet at δ 24·5 is distinguished from that at δ 38 since C-4 in all the other molecules has the highest shift. The two remaining NCH_2 groups were assigned by considering that the absorption for the benzylic methylene group was always at lower field than the other CH_2 groups bonded to the nitrogen atom. The doublet at lowfield (δ 73) was assigned to C-3 by analogy with the situation in montanine and tazettine.[1] Of the three remaining CH groups, the C-5a has a shift comparable with that in crinine; however C-1 and C-2 are indistinguishable. Finally the two methoxy-groups have a very small shift difference which precludes precise assignment.

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