Tetrahedron Vol. 43, No. 1, pp 149 to 157, 1987
Printed in Great Britain.

0040-4020/87 $5.00+.00

THE DETERMINATION OF THE MOLECULAR FRAMEWORK AND CONFORMATION OF THE CALPAURINE ALKALOID BY 1D- AND 2D- NMR METHODS

Paolo Mascagni[1*], William A. Gibbons[1*], Kaleab Asres[2], J. David Phillipson[2], and Neri Niccolai[3]

1 Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX.

2 Department of Pharmacognosy, School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX.

3 Department of Chemistry, University of Siena, 53100 Siena, Italy.

(*Received in UK* 3 *November* 1986)

Abstract - The structure and conformation of calpaurine, a novel quinolizidine alkaloid, has been studied and found to be that of the 3(β),4(α)-dihydroxy,13(α)-O-(2'-pyrrolylcarbonyl) derivative of lupanine.

The approach used for building the molecular framework is based on the use of mono- and bi-dimensional NMR technique and the knowledge of the molecular formula of calpaurine.

INTRODUCTION

The genus Calpurnia (Leguminosae) comprises 6 or 7 species which occur in several African countries[1]. Calpurnia aurea (Ait.) Benth. extends from Eritrea to Cape Province and is also found in S. India. Subspecies aurea from Ethiopia, known locally as "Digitta", is used in indigenous medicine for the treatment of diarrhoea and skin complaints, as well as being employed as an insecticide[2]. Previous chemical investigations of C. aurea has resulted in the isolation of a series of quinolizidine alkaloids[3]. Investigation of the leaves of Ethiopian C. aurea has been extended and four new alkaloids isolated[4,5]. In this communication the application of modern NMR techniques to the elucidation of structure of one of the new alkaloids calpaurine 1 is described. This work illustrates the rapid and powerful use of 1- and 2D-NMR methodologies for solving natural product problems which previously would have been resolved by X-ray studies.

To illustrate the use of NMR methods for rapid determination of the molecular framework and conformation of natural products, we have assumed only two facts: (i) the molecule was a pyrrole carboxylic ester and (ii) had a molecular formula $C_{20}H_{27}N_3O_5$. Since only 10 mg was available it was not possible to undertake ^{15}N-NMR studies, which would have made the first assumption unnecessary. IR, mass spectroscopy and other data[4] were used solely to confirm our conclusions concerning the molecular framework and conformation.

RESULTS AND DISCUSSION

The proton spectrum of calpaurine in $CDCl_3$ at 300 MHz is shown in figure 1. The broad signal centred at 3.1 ppm readily disappeared upon addition of D_2O, suggesting that acidic moieties are present in the molecule; a second signal at 9.35 ppm, also exchanged with D_2O although at a much slower rate (< 1 hr). The integral of the 500 and 300 MHz spectra of the D_2O exchanged sample yielded 24 protons consistent with three acidic moieties in calpaurine. The scalar couplings between protons were assigned by means of 2D homonuclear shift correlation spectroscopy. The analysis of the intensity contour map, permitted the grouping of the proton signals into four sets of mutually-coupled spins, schematically illustrated in Figure 2; scalarly coupled protons are joined by solid lines.

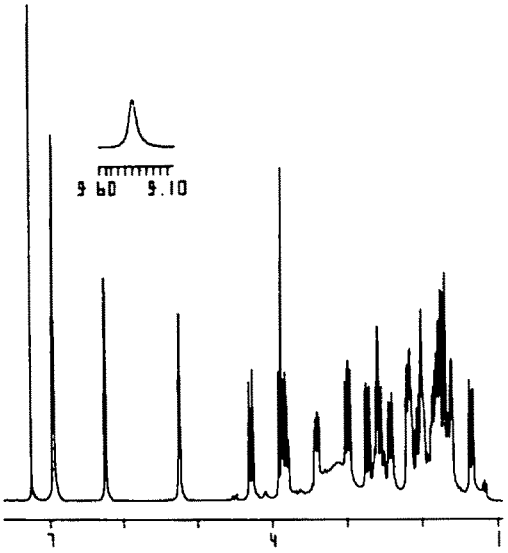

Figure 1. 300 MHz ^{1}H-NMR spectrum of calpaurine in $CDCl_3$.

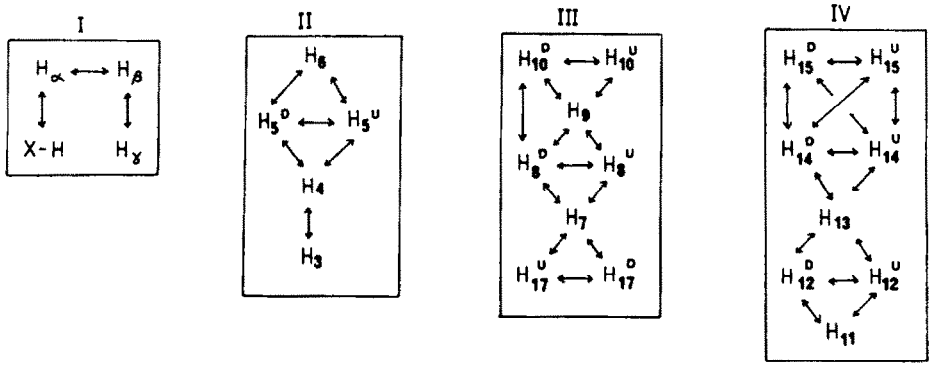

Figure 2. ^{1}H-^{1}H chemical shift correlations obtained from the analysis of a COSY spectrum of calpaurine in $CDCl_3$.

Chemical shift considerations readily assigned the protons at 6.97 (two Hs) and 6.29 ppm (Hα, Hδ, Hβ in box I of Figure 2) to the three aromatic protons of the pyrrole 2'-carboxylate and the exchangable proton at 9.53 ppm to its N-H group. These conclusions were later confirmed by the

heteronuclear correlation NMR and by the spectrum of the calpaurine hydrolysate which lacked the aforementioned four signals.

At this stage we made no attempt to distinguish ^{2}J, ^{3}J or long range coupled spins in the three other spin-connectivities boxes of Figure 2.

The broad-band decoupled ^{13}C spectrum of calpaurine in $CDCl_3$ yielded the 20 carbon signals expected from the molecular formula (Figure 3a).

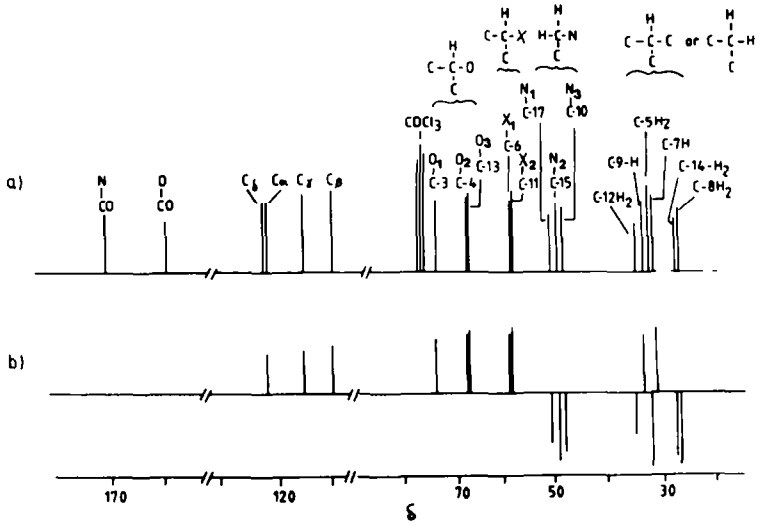

Figure 3. a) 75 MHz broad-band decoupled ^{13}C spectrum of calpaurine in $CDCl_3$ and fragment assignments (see text).
b) Trace of the D.E.P.T. spectrum of calpaurine in $CDCl_3$.

The DEPT experiment (Figure 3b) showed that three of these signals were of non-protonated carbons, two of which were assigned to the C-α carbon of pyrrole and its attached carbonyl carbon. This left seventeen protonated and one unprotonated carbon for the molecular framework to be assigned.

The CH_n (n=1,2,3) groups were then divided into ten CH_3/CH signals ("up" in the DEPT spectrum) and seven CH_2 signals ("down"). Since the latter accounted for fourteen of the twenty-four attached protons, this proved that each of the ten remaining carbons had to be CH.

In general the 2D carbon-proton correlation experiment confirmed these conclusions and correlated the proton chemical shifts with those of their attached carbon atoms. Thus in the hetero-correlation spectrum all CH_2 groups identified in Figure 3 yielded both an upfield and a downfield proton with the exception of the CH_2 group at position 15 which had essentially degenerate proton chemical shifts. There was complete agreement between the detection of CH groups in Figure 3 and in the hetero-correlation experiment.

At this stage we therefore had a total carbon and proton (unexchangable ones) count from NMR that agreed with the molecular formula, plus a correlation between the chemical shifts of directly bonded protons and carbons. As material was strictly limited it was impossible to undertake other 2D experiments such as long range hetero COSY, carbon-carbon connectivity[6] and proton-carbon nOes, which would have made possible to propose at least a partial molecular framework.

Substituent effects were then used to assign the oxygen and nitrogen atoms to the individual carbons of the CH and CH_2 groups. Thus, (i) six upfield ^{13}C signals from 34.6 ppm to 26.5 ppm were assigned either to CH or CH_2 groups with no directly bonded oxygen or nitrogen atoms; (ii) the two downfield signals had no protons directly attached and were assigned to the pyrrole carbonyl (171.8 ppm) and to a carbonyl whose location was not fully assigned at this stage (160.8

ppm); (iii) the four pyrrole ring carbons between 110 and 125 ppm were readily assigned; (iv) the C-3, C-4 and C-13 atoms were of the form C -- CH(C) -- O; (v)the C-10, C-15 and C-17 atoms were of the type C -- CH_2 -- N. This left two carbon atoms, C-6 and C-11, as yet unassigned. It was not possible to designate them unequivocally as C -- CH(C) --N or C -- CH -- O and hence we designated them C -- CH(C) -- X.

To place the carbon, nitrogen and oxygen atoms in their appropriate molecular locations, we correlated the data in Figures 2 and 3. This was achieved as follows: (a) C-5, C-10, C-8, C-17, C-15, C-14, and C-12 were readily inserted into the boxes of Figure 2 to yield the geminal CH_2 group of Figure 4; (ii) the CH groups C-4, C-3, C-9, C-7, C-13 and C-11 were inserted in a similar manner.

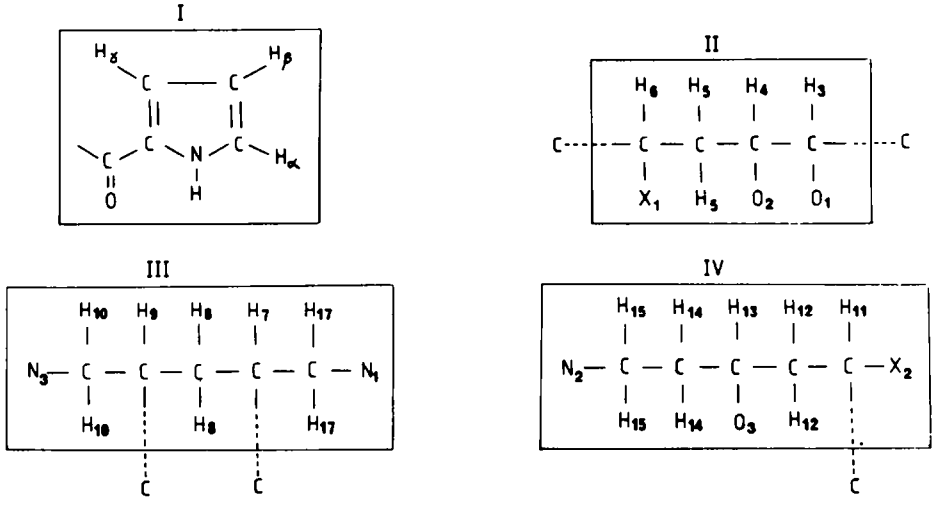

Figure 4. Assignment of "fragments" of calpaurine, obtained by combining the data from the COSY experiment and that from the ^{13}C-NMR experiments.

A significant step in this procedure was then achieved by recognizing that the two protons that exchanged with addition of D_2O also gave nOes at the protons at C-3 and C-4. This allowed designation of the functionalities at C-3 and C-4 as tertiary alcohols and provided a total proton and oxygen accounting. The corrollary to this is that X_1 and X_2 in Figure 4 had to be nitrogen atoms and hence the molecule had to possess five carbons attached directly to two tertiary nitrogens.

Proton-proton through-space connectivities obtained by 1D and 2D measurements were divided into "intrabox" and "interbox" as depicted in Figures 5 and 6 respectively. Those in Figure 6 clearly showed that the methylene protons at C-17 and C-15 were connected via a C-17 -- N -- C-15 linkage. Since only one nitrogen remained unassigned and there were no through-space proton connectivities between boxes II and IV but a large number between II and III, it was postulated that $N_3 = N_4$ and the function is C-6 -- N(C) -- C-10.

Molecular modelling based upon all of the "interbox" nOes produced the molecular framework of Figure 7a. This meant that $N_1 = N_2 = N_5$ and the tertiary function was C-17 -- N(C-11) -- C-15. The C-13 -- O bond was assigned to the pyrrole carboxylic ester: all that remained was the unassigned low field ^{13}C carbonyl group and one unassigned tertiary nitrogen valence. These were accounted for by the lactame bond in ring A of Figure 7b, thus establishing calpaurine as a member of the family of sparteine alkaloids.

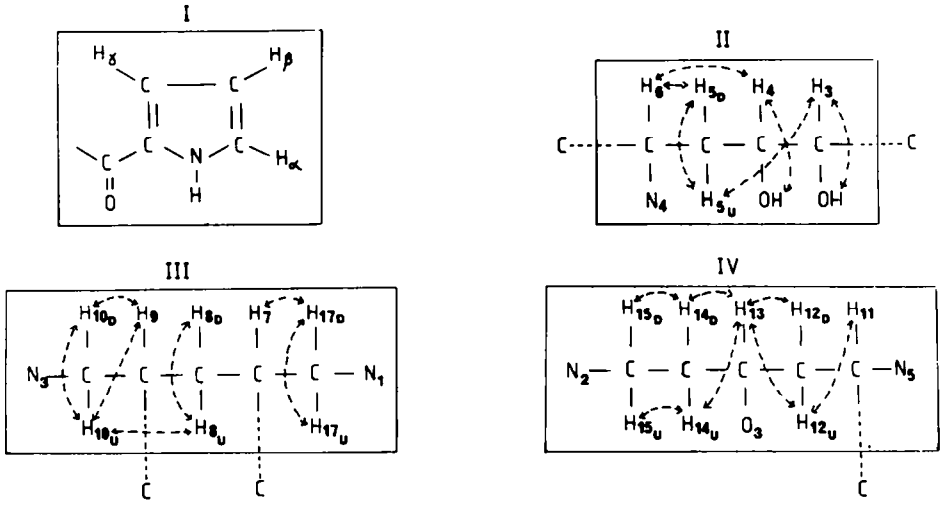

Figure 5. "Intraboxes" NOEs showing the dipolar interactions within individual fragments of calpaurine. Cf. figure 4.

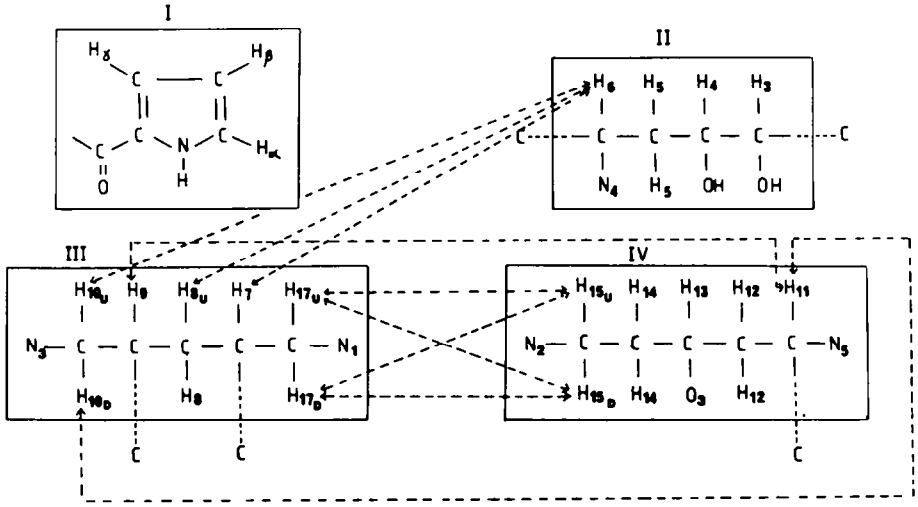

Figure 6. "Interboxes" NOEs between "fragments" of calpaurine. Cf. figures 4 and 5.

At this stage we had no need to use the "intrabox" geminal, vicinal nOes or those between atoms five or more bonds apart. These are shown in Figure 5 and were fully consistent with the molecular formula of Figure 7b.

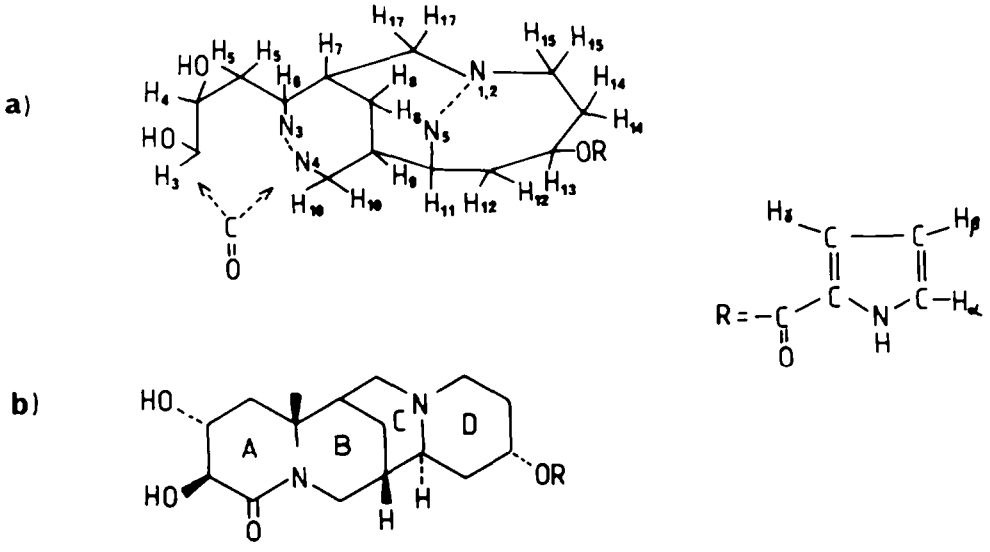

Figure 7. a) Incomplete structure of calpaurine.
b) Complete structure of calpaurine obtained using the NMR data discussed in the text and molecular modelling.

Distinction between 2-oxosparteine and isosparteine-type compounds was based on the Overhauser effect. Thus the nOe recorded at H-10(d) after irradiation of H-11 indicated that the latter proton was alpha, proving that calpaurine possesses the structure of 2-oxosparteine. Additional evidence for the latter statement came from the comparison of the ^{13}C chemical shifts of 1 with those reported in the literature for α-isolupanine and lupanine compounds[7]. As it appears from Table I significant differences, clearly attributable to the conformation of H-11, exist for the chemical shift of C-8, C-11, C-15 and C-17. These resonances for calpaurine were virtually identical with those of the hydroxy lupanines (2-oxo-13-hydroxysparteine) hence confirming the previous assignment.

Conformational analysis of calpaurine

a) From nOe data

The nOes observed at H-8(u), H-10(u), H-5(d) and H-4 upon irradiation of H-6 (see text above and Figure 5) were consistent with the following: (i) ring B is in the chair conformation, (ii) H-8(u) and H-10(u) are both axial and (iii) H-5(d) is above the plane containing ring A. Furthermore they established that the OH group at C-4 has an alpha configuration. The other hydroxyl group, that at C-3, was found to be equatorial and beta since H-3 and H-5(u) were dipolarly coupled.

The lack of effects on H-11 and on either two protons at C-15, after irradiation of H-13 indicated that the O-pyrrole moiety at C-13 is axial. Thus molecular models showed that there would be Overhauser effect at H-11 and at the H-15 with the axial conformation if H-13 were also axial. This assignment was confirmed by the ^{13}C spectrum of the alcohol derivative of calpaurine.

TABLE I: Comparison of the ^{13}C chemical shifts[1] of calpaurine (I), its alcohol derivative (II) and some hydroxysparteines[2].

	I	II	III	IV	V
C-2	171.8	173.8	-	-	-
C-3	74.3	75.6	32.9	33.0	33.1
C-4	68.0	69.6	19.6	19.6	19.8
C-5	26.5	27.1	26.6	26.7	27.8
C-6	57.7	59.3	60.8	58.7	58.7
C-7	33.4	35.3	34.2	34.5	35.2
C-8	27.5	31.8	27.3	27.4	35.3
C-9	32.2	33.4	31.6	32.6	34.5
C-10	48.3	49.9	46.8	46.9	42.3
C-11	57.4	58.7	57.0	61.3	63.3
C-12	34.6	39.9	39.9	41.5	40.1
C-13	68.4	65.3	64.0	69.6	69.6
C-14	32.4	34.2	32.4	33.8	34.2
C-15	49.4	50.3	49.2	51.5	55.0
C-17	50.5	53.0	52.4	53.0	56.1
C-18	160.8	-	-	-	-
C-19	122.9	-	-	-	-
C-21	123.4	-	-	-	-
C-22	110.3	-	-	-	-
C-23	116.1	-	-	-	-

1) chemical shifts are in ppm and refer to TMS as internal standard
2) the chemical shifts for the hydroxysparteines were taken from reference 8.
III = 13-α-hydroxylupanine; IV = 13-β-hydroxylupanine; V = 13-β-hydroxyisolupanine

The chemical shift of C-13 was at 65.3 ppm, virtually identical to those of other 13-α-hydroxylupanines and substantially lower than that of 13-β-hydroxylupanines (Table I).

Other configurations were established using the nOes from Figures 5 and 6 and the results thus obtained are summarised in Table I.

TABLE II: ^1H-^1H coupling constants of calpaurine

^1H-^1H	J (Hz)	^1H-^1H	J (Hz)
3-4	9.53	13-14(d)	2.5 or 0.3
4-5(d)	4.75	13-14(u)	0.3 or 2.5
4-5(u)	11.80	14(u)-14(d)	-
5(d)-6	5.27	14(d)-15(d)	0.9
5(u)-6	11.7	14(d)-15(u)	12.56
5(u)-5(d)	13.3	14(u)-15(d)	3.0
6-7	1.48	14(u)-15(u)	2.0
7-17(d)	8.34	15(u)-15(d)	12.6
7-17(u)	4.00	Hα-Hβ	3.5
17(u)-17(d)	11.91	Hβ-Hδ	2.7
7-8(d)	0.6		
7-8(u)	2.3		
8(d)-8(u)	12.4		
8(d)-9	1.9		
8(u)-9	2.3		
8(d)-10(d)	1.99		
9-10(d)	1.99		
9-10(u)	3.0		
9-11	1.0		
10(d)-10(u)	13.46		
11-12(d)	11.91		
11-12(u)	1.0		
12(u)-12(d)	14.04		
12(d)-13	2.5 or 0.3[1]		
12(u)-13	0.3 or 2.5[1]		

1) It was not possible from the present data to distinguish between the two possibilities.

b) From J coupling constants

The coupling constants of all the protons, with the exception of the geminal value for protons 14, were assigned either directly from the NMR traces and then refined by spectral simulation, or by spectral simulation only(Table II).

The assignment of the proton configurations from coupling constants is shown in Table III where they are directly compared with the configurations derived from nOe data. No discrepancies

were found between these two sets of data hence indicating that the compound had the structure of 3β,4α-dihydroxy 13α-O-(2'-pyrrolylcarbonyl)-lupanine.

TABLE III: ^{1}H chemical shift and proton configuration assignments of calpaurine in $CDCl_3$ solution.

	δ (ppm)[2]	configuration from nOe data	configuration from J values
H-3	3.92	a (α)	a -
H-4	3.84	a (β)	a -
H-5(d)	2.06	e (β)	e (β)
H-5(u)	1.77	a (α)	a (α)
H-6	3.42	a (β)	a (β)
H-7	2.01	e (β)	- -
H-8(d)	2.17	e (α)	e (α)
H-8(u)	1.38	a (β)	e (β)
H-9	1.64	e (β)	e (β)
H-10(d)	4.30	e (α)	e (α)
H-10(u)	2.74	a (β)	a (β)
H-11	2.44	a (α)	a (α)
H-12(d)	1.85	a (β)	a (β)
H-12(u)	1.71	e (α)	e (α)
H-13	5.25	e (β)	e (β)
H-14(d)	1.86	a (β)	a (β)
H-14(u)	1.77	e (α)	e (α)
H-15(d)	2.62	e (β)	e (β)
H-15(u)	2.56	a (α)	a (α)
H-17(d)	3.00	e (β)	- -
H-17(u)	2.20	a (α)	- -
H-α	6.97	- -	- -
H-δ	6.97	- -	- -
H-β	6.29	- -	- -
N-H	9.53	- -	- -

1) a = axial; e = equatorial; β = above the plane containing the ring; α = below the same plane.
2) chemical shifts refer to TMS.
3) d = downfield in the proton spectrum; u = upfield in the spectrum.

CONCLUSIONS

In the present study we have described the structure and conformational analysis of a novel alkaloid of the family of the oxosparteines. The analysis was carried out using exclusively NMR observations from mono and two-dimensional techniques. The latter permitted to build up fragments of the backbone of the alkaloid of which only the molecular formula was initially known. In a puzzle-type fashion, chemical considerations supported by molecular modelling and NMR observations led to the conclusive structure of calpaurine (Figure 7b). The relevance of the data here reported and in particular the approach used for the determination of the structure and conformation of the alkaloid illustrates, in our opinion, how the combined use of (i) high resolution NMR spectrometers (ii) the application of modern NMR methodologies for the assignment of parameters such as scalar and dipolar couplings, chemical shifts etc and (iii) chemical intuition can be used for solving the structure of unknown natural products.

EXPERIMENTAL

The isolation and purification of calpaurine is described elsewhere[4].

10 mg of calpaurine was dissolved in 0.5 ml of $CDCl_3$ and the solution thus obtained used for, both the ^{13}C and ^{1}H NMR measurements. A Varian XL-300 spectrometer was used for the majority of the experiments. Some decoupling experiments and an integrated spectrum of calpaurine were performed on the Bruker AM-500 spectrometer at the National Institute of Medical Research at the Ridgeway, Mill Hill, London.

For the ^{13}C-^{1}H chemical shift experiment the phase cycling pulse described by Bax et al.[8] was used. The classical 90-t-90-acq and the 90-t-90-mix-acq (mix=0.35 s) pulses were used for the proton-proton chemical shift and NOESY experiments respectively[9,10]. Proton resonances were saturated for 5 seconds prior to acquisition during the mono-dimensional nOe experiments.

REFERENCES

1. R.K. Brummit, Kirkia, 1976, 6, 123.
2. P.C.M. Jansen, Belmontia, 1981, 12, 155 and references therein.
3. J.L. Van Eijk and M.H. Radema, Planta Medica, 1977, 32, 275.
4. K. Asres, W.A. Gibbons, J.D. Phillipson and P. Mascagni, Phytochemistry, 1985, in press.

5. K. Aares, J.D. Phillipson and P. Mascagni, Planta Medica, 1986. in press.
6. A. Bax, R. Freeman and S.P. Kempsell, J. Am. Chem. Soc., 1980, 102, 4849.
7. F. Bohlmann and R. Zeisberg, Chem. Ber., 1975, 108, 1043,
8. A. Bax and G.A. Morris, J. Magn. Reson., 1981, 42, 501.
9. W.P. Aue, E. Bartholdi and R.R. Ernst, J. Chem. Phys., 1976, 64, 2229.
10. S. Macura and R.R. Ernst, Mol. Phys., 1980, 41, 95.