Tetrahedron Letters No. 51, pp 5081 - 5084, 1973. Pergamon Press. Printed in Great Britain.

STRUCTURE ANALYSIS BY ^{13}C NMR SPECTROSCOPY OF CRIOPHYLLINE, A NEW DIMERIC INDOLE ALKALOID[1]*

A. Cavé, J. Bruneton
(U.E.R. Chimie Thérapeutique. Centre d'Etudes Pharmaceutiques de Châtenay, 92290 - Châtenay-Malabry, France)

A. Ahond, A.-M. Bui, H.-P. Husson, C. Kan, G. Lukacs and P. Potier
(Institut de Chimie des Substances Naturelles du C.N.R.S. 91190 - Gif-sur-Yvette, France)

(Received in UK 19 October 1973; accepted for publication 8 November 1973)

We wish to report on the structure analysis by ^{13}C nmr of criophylline 1, a new dimeric indole alkaloid, [mp 278-280° ; $[\alpha]_D^{22}$ - 176° (c 1.04 $CHCl_3$) ; m/e 646.3528 (calc 646.3519) $C_{40}H_{46}N_4O_4$, isolated from the leaves of Crioceras dipladeniiflorus (Stapf.) K. Schumann. The elucidation of this highly complex structure is based almost completely on ^{13}C nmr data[2]. ^1H nmr and mass spectral analysis provided complementary informations about the constitution of this alkaloid[3].

Criophylline does not undergo acetylation under normal conditions. Its 100 MHz pmr spectrum indicates a carbomethoxy function [δ 3.72(s)] , two methyl groups on secondary carbon centers [0.9(t, J = 6.5 Hz)] , seven unsaturated hydrogens and several overlapping uninterpretable multiplets. The uv spectrum [EtOH, λ nm, (log ε)] : 214(4.26), 228(4.06), 257(3.93), 299(4.08), 327(4.28) consists of the addition of two chromophores : one of tabersonine type[4], the other of dihydroindole type. The mass spectrum [m/e 432.2650 (calc 432.2651 $C_{27}H_{34}O_2N_3$)] reveals retro Diels-Alder fragmentation, typical of a tabersonine-like moiety[4].

Noise and single frequency decoupled ^{13}C nmr spectra[5] of criophylline shows below 80 ppm : four nonprotonated carbons, eight methines, ten methylenes and three methyl groups. Above 80 ppm eight quaternary and seven methine carbons can be observed in agreement with the 46 hydrogen atoms of the molecule, suggesting that each of the indole nitrogens bears a hydrogen.

The high number of saturated methine sites (eight) with respect to only one in tabersonine and their relatively high chemical shifts for alco-

(*) Dedicated to Professor M.-M. Janot on his 70th birthday.

holic oxymethine carbons suggested epoxide linkages for the two unaccounted oxygens. Comparison of the ^{13}C nmr data of hazuntinine 2[6] and the new dihydroindole alkaloid andrangine 3[7] with the spectrum of criophylline afforded proof that the individual components of the dimer were related to these two epoxy-alkaloids.

While each saturated andrangine carbon has its signal unaffected in the ^{13}C nmr spectrum of criophylline (both from the point of view of chemical shift and single frequency decoupled multiplicity ; see Table and Figure) some hazuntinine carbons below 80 ppm are markedly influenced by the linkage of the two monomers. Inspection of the unsaturated carbon region of the criophylline spectrum indicates an unsubstituted aromatic ring for the indole moiety[6] while one aromatic protonated carbon of the andrangine part becomes a quaternary site. As a consequence, the latter unsaturated dihydroindole carbon - assigned to C-10 [6,8] - must be attached to a saturated site of the epoxy-tabersonine moiety. As the single frequency decoupled spectrum of criophylline exhibited onIy three aminomethylene signals, either C-5' or C-3' must be the second site of linkage*.

The lowest field carbon which can be assigned to C-21' appears at 62.0 ppm. Due to the shielding of this signal with respect to C-21' of hazuntinine (70.8 ppm) H-21' must be involved in a strong steric interaction. Either the C-14' - C-15' epoxide in the dimer is on the same α side as H-21' or the linkage of the monomers takes place at the α side at C-5' or C-3', hydrogen-21' being then involved in a 1,3-type diaxial interaction[9]. The almost identical C-17' and C-19' shifts for the monomer and the dimer do not support the first hypothesis.

The ^{13}C nmr spectrum of criophylline offers at first sight two different interpretations : in case of a substitution on C-5' α** : (δ monomer-dimer) C-3' 49.2 - 42.4 (peri interaction) ; C-5' 51.4 - 58.1 (α effect) ; C-6' 43.4 - 48.4 (β effect) ; C-14' 51.8 - 54.2 (?) ; C-15' 57.0 - 56.5 ; C-21' 70.8 - 62.0 (1,3-interaction).

(*) The prime symbols are applied for convenience to the epoxytabersonine carbons, both in the monomer and the dimer.
(**) Carbons not discussed have identical shifts in the monomer and in the dimer (Table).

TABLE

^{13}C NMR CHEMICAL SHIFTS[5] OF CRIOPHYLLINE 1, ANDRANGINE 3 AND EPOXY-14, 15β-TABERSONINE 4[c]

	C-2'	C-3'	C-5'	C-6'	C-7'	C-8'	C-9'	C-10'	C-11'	C-12'	C-13'
1	165.0	58.1	48.4	42.4	54.2	138.0	121.5	120.2	127.6	109.1	143.1
4	166.0	49.2	51.4	43.4	54.8	138.0	121.0	120.5	127.4	109.3	143.4

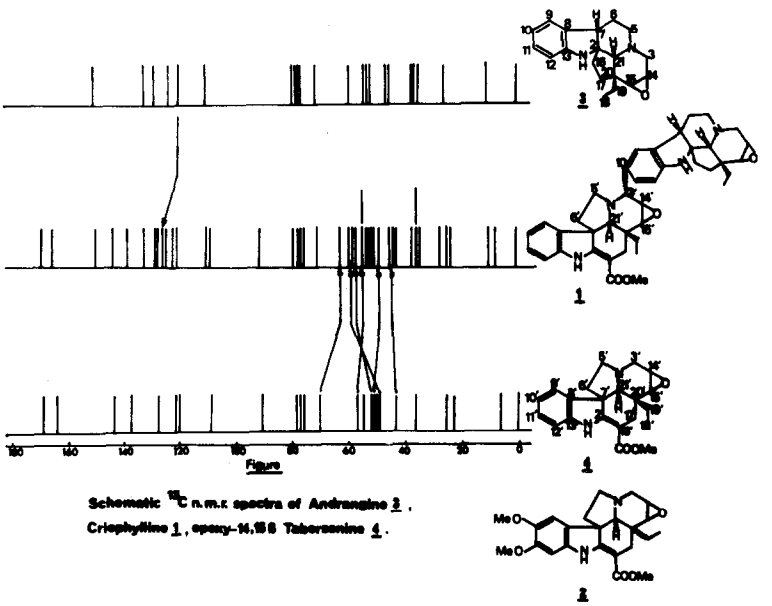
	C-14'	C-15'	C-16'	C-17'	C-18'	C-19'	C-20'	C-21'	C=O'	OMe'
1	56.8	54.2	90.8	23.9	7.3	27.0	36.6	62.0	168.8	50.9
4	51.8	57.0	90.7	23.3	7.0	26.3	36.8	70.8	168.8	50.7

	C-2	C-3	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13
1	75.9	50.6[b]	52.1[b]	24.2	44.1	132.0	123.9	125.2	127.6	108.6	149.1
3	75.3	50.4[b]	51.5[b]	24.2	44.2	131.8	122.7	118.9	127.4	109.3	149.5

	C-14	C-15	C-16	C-17	C-18	C-19	C-20	C-21
1	51.5	58.3	34.3[a]	35.0[a]	9.7	35.0[a]	43.9	70.2
3	51.9	58.3	34.2[a]	34.9[a]	9.6	35.2[a]	43.8	70.1

(a)(b) Assignment may be reversed in horizontal lines.

(c) Chemical shifts are those observed for hazuntinine 2, for the six aromatic carbons δ values of tabersonine have been given[6].



Figure

Schematic ^{13}C n.m.r. spectra of Andrangine 3, Criophylline 1, epoxy-14,15β Tabersonine 4.

In case of a substitution on C-3'α : C-3' 49.2 -58.1 (α effect) ; C-5' 51.4 - 48.4 (peri interaction); C-6' 43.4 - 42.4 ; C-14' 51.8 - 56.8 (β effect) ; C-15' 57.0 - 54.2 (1,3-interaction half-chair conformation) ; C-21' 70.8 - 62.0 (1,3-diaxial interaction).

The substitution on C-5'α can be hardly reconciled with the variation of the shift of C-14' and the shielding of C-3' seems to be too strong.

Inspection of the ^{13}C nmr spectra of a variety of steroidal epoxide models[9] supports well the 2.8 ppm shielding effect of C-15' upon C-3'α substitution. The agreement between expected and experimental values is much better in this case. While the assignment of the structure is on firm ground, that of the substitution site of the epoxy-tabersonine moiety at C-3' is only tentative at this time.

REFERENCES

1. Résonance Magnétique Nucléaire du ^{13}C de Produits Naturels et Apparentés XV. for paper XIV. see A. Ahond, M.-M. Janot, N. Langlois, G. Lukacs, P. Potier, P. Rasoanaivo, M. Sangaré, N. Neuss, M. Plat, J. Le Men, E. Hagaman and E. Wenkert, J. Amer. Chem. Soc., in press.
2. For other reports on structure analysis of alkaloids where ^{13}C nmr played a major role see : a) A. Rabaron, M. Koch, M. Plat, J. Peyroux, E. Wenkert and D. Cochran, J. Amer. Chem. Soc., 1971, 93, 6270 ; b) E. Wenkert, C.-J. Chang, D.W. Cochran and R. Pellicciari, Experientia, 1972, 28, 377; c) A. Jones and M.H. Benn, Tetrahedron Letters, 1972, 4351 ; d) J. Le Men G. Lukacs, L. Le Men-Olivier, J. Lévy and M.-J. Hoizey, Tetrahedron Letters, in press.
3. J. Bruneton, Thèse de Doctorat ès Sciences, Université de Paris-Sud, Centre d'Orsay, 1973.
4. M. Plat, J. Le Men, M.-M. Janot, J.M. Wilson, H. Budzikiewicz, L.J. Durham, Y. Nakagawa and C. Djerassi, Tetrahedron Letters, 1962, 271.
5. Spectra have been recorded in $CDCl_3$ solution at 22,63 MHz on a Bruker HX 90E Fourier transform spectrometer, chemical shifts of the Table are given with respect to TMS = 0, used as an internal standard.
6. E. Wenkert, D.W. Cochran, E.W. Hagaman, F.M. Schell, N. Neuss, A.S. Katner, P. Potier, C. Kan, M. Plat, M. Koch, H. Mehri, J. Poisson, N. Kunesch and Y. Rolland, J. Amer. Chem. Soc., 1973, 95, 4990.
7. The structure of andrangine has been elucidated recently by chemical correlations : **C. Kan, B.C. Das, H.-P. Husson and P. Potier**, unpublished results. Analysis of the ^{13}C nmr spectrum of this alkaloid will be discussed elsewhere.
8. R.G. Parker and J.D. Roberts, J. Org. Chem., 1970, 35, 996.
9. The β configuration has been assigned tentatively[6] to the epoxide of hazuntinine. This assumption is supported by a systematic study on the ^{13}C spectra of steroidal epoxides : K. Tori, T. Komeno, M. Sangaré, B. Septe, A. Ahond and G. Lukacs, unpublished results.