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^{13}C NMR SPECTROSCOPY OF CUCURBITACINS

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Abstract—The ^{13}C NMR spectra of nine representative cucurbitacins have been analysed for signal assignment, by deduction and selective comparison. Detailed ^{1}H NMR data have been provided.

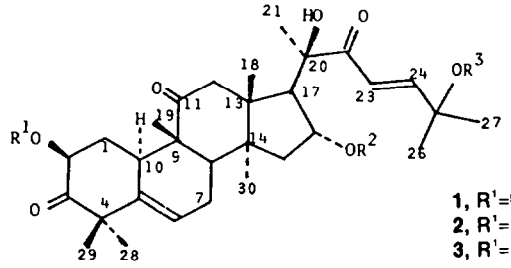
The cucurbitacins are a group of highly oxygenated tetracyclic triterpenes having a cucurbitane skeleton characterized by a 19(10→9β) *abeo*-10α-lanostane. This group of compounds has been studied extensively chemically,[1] and an X-ray structure analysis has been reported[2] on a di-*p*-iodobenzoate ester of cucurbitacin D. A number of compounds of this group have been investigated for their cytotoxic properties,[3,4] antifertility activity[5] and as plants growth regulators.[6] There seems to be a renewed interest in this class of compounds and a ^{13}C NMR analysis of a number of basic cucurbitacins should provide physical data of importance for future research in this field.

Previous isolations, identifications and chemical reactions performed in our laboratory on several cucurbitacins, led to the accumulation of a number of structurally closely related compounds which made them good substrates for ^{13}C NMR analysis. The spectroscopic data collected for compounds **1–9** are reported in Table 1, and the most characteristic features are discussed in the sequel. The attribution of the signals as δ, obtained from proton decoupled and single-frequency off-resonance decoupled spectra (SFORD), was based on the analogy of the different groups and the known effect of substituents. For correlation of the various residual ^{13}C-H coupling constants to the ^{1}H NMR chemical shifts, all proton spectra were recorded for consistent data on the same instrument. Furthermore, in view of a careful selection of the different compounds studied, which differed from one another at specific sites, it was possible to provide an unambiguous assignment for each of the eight Me groups and the relevant protons. For example, by comparing **5** to **6** and **7** to **8**, the assignment of the 26,27-Me, due to the 20-OAc (in **6** and **8**), was

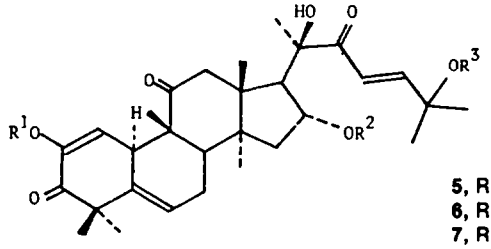
Table 1. Carbon shifts of cucurbitacins **1–9**†

Carbons	1	2	3	4	5	6	7	8	9
1	36.0	32.0	36.0	32.0	115.0	115.2	131.7	131.5	115.0
2	71.7	73.3	71.6	73.2	144.6	144.8	143.4	143.3	144.7
3	212.4	205.7	212.4	205.6	198.7	198.9	195.0	194.9	198.7
4	50.3	48.4	50.3	48.8	47.6	47.7	48.1	48.0	47.6
5	140.6	139.8	140.5	139.8	137.0	136.6	136.2	136.1	136.9
6	120.3	120.4	120.4	120.2	120.7	120.6	121.4	121.3	120.7
7	24.0	23.7	23.9	23.9	23.6	23.5	23.5	23.7	23.6
8	42.5	42.1	42.4	42.6	41.6	41.6	41.3	41.2	41.6
9	48.4	48.1	48.4	48.4	48.8	48.9	48.4	48.4	48.8
10	33.8	34.3	33.8	34.3	34.7	34.6	35.8	35.7	34.7
11	213.1	211.9	213.1	210.8	212.9	214.0	212.6	212.4	213.0
12	48.8	48.6	48.7	46.9	48.8	48.6	48.9	48.8	48.8
13	48.3	50.0	48.4	49.4	48.3	48.0	49.9	49.8	48.3
14	50.9	51.3	50.8	51.3	50.8	50.4	51.4	51.4	50.8
15	45.6	43.1	45.4	43.3	45.7	45.3	43.4	43.3	45.6
16	71.5	73.7	70.9	74.7	71.6	70.8	73.7	73.5	71.0
17	57.3	54.0	57.8	63.8	57.4	58.0	54.2	54.2	57.8
18	20.1	19.8	19.8	19.8	20.0	19.8	19.8	19.7	19.8
19	19.3	18.9	18.9	18.7	18.6	17.6	18.3	18.1	18.3
20	78.2	77.7	79.2	206.0	78.1	78.5	77.7	77.7	79.2
21	24.0	23.6	24.5	31.3	24.0	23.8	23.7	23.5	24.5
22	202.7	201.3	215.6	-	202.7	203.1	201.3	200.9	215.4
23	119.1	118.5	30.9	-	119.0	120.6	118.6	119.3	30.9
24	156.0	155.5	37.0	-	155.9	151.3	155.7	152.7	37.0
25	71.2	71.3	70.3	-	71.2	79.6	71.2	79.1	70.3
26	28.9*	29.5*	28.7*	-	29.0*	26.0	29.6*	26.6*	28.7*
27	29.6*	29.7*	29.9*	-	29.6*	26.0	29.7*	26.3*	29.9*
28	21.3	21.3	21.3	21.3	20.1	19.7	20.3	20.2	20.2
29	29.4	28.6	29.3	28.6	27.9	27.8	27.2	27.2	27.9
30	20.1	20.0	20.0	20.0	20.1	20.0	20.2	20.2	20.1
2-OAc	-	20.7	-	20.6	-	-	20.3	20.6	-
	-	170.1	-	170.1	-	-	169.0	168.9	-
16-OAc	-	20.7	-	21.1	-	-	20.7	20.6	-
	-	170.1	-	170.5	-	-	170.1	169.6	-
25-OAc	-	-	-	-	-	21.7	-	21.8	-
	-	-	-	-	-	171.6	-	170.3	-

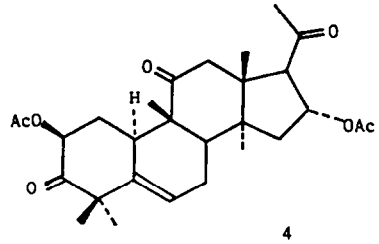
†In ppm downfield from TMS; $CDCl_3$ solutions containing TMS as standard.
*Signals which in any vertical column may be interchanged.



1, $R^1=R^2=R^3=H$
2, $R^1=R^2=Ac$, $R^3=H$
3, $R^1=R^2=R^3=H$, 23,24-dihydro



5, $R^1=R^2=R^3=H$
6, $R^1=R^2=H$, $R^3=Ac$
7, $R^1=R^2=Ac$, $R^3=H$
8, $R^1=R^2=R^3=Ac$
9, $R^1=R^2=R^3=H$, 23,24-dihydro



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unequivocal. Other deductions and comparisons can be readily made by using the data collected in Table 2 provided for further reference.

The *quarternary carbons* C-4, 9, 13 and 14 could be recognized by the unchanged values observed for the C-4 and 9, following modifications in the side chain; alternatively, the C-13 and 14 were not affected when changes were introduced in ring A. This can be well visualized by examining Fig. 1, part A. Indeed, when compound **2** is compared to its corresponding hexanor derivative **4**, in which the side chain has been removed, only C-13 exhibits an upfield shift. Inversely, when in ring A the α-ketol is replaced by a diosphenol, **1** to **5**, only C-4 undergoes an upfield shift, whereas C-9 moves slightly downfield. All four quarternary carbons were found, as expected, not to be influenced by the hydrogenation of the 23,24-double bond, compare **1** with **3** and **5** with **9**.

The three *methine carbons* C-8, 10 and 17, being under the influence of a γ-effect related respectively to the 18, 28 and 30-Me groups, their assignments were made by the correlation existing between the ^{13}C–H coupling constant and the corresponding ^1H NMR chemical shifts. In the ^1H NMR spectrum of cucurbitacin I (**5**) (Table 2), the positions of 10 and 17-H were determined unequivocally by double irradiation and found to be sufficiently separated, δ 3.55 and 2.54, to provide unambiguous correlation with their J ^{13}C-H values. Moreover, confirmation of the allocation of the C-17 signal (δ 57.3 in **1**) was obtained by comparing the following three pairs of compounds. In the first pair, **1** vs **2**, a 3.3 ppm upfield shift is observed after acetylation of the 16-OH group; in the second pair, **1** vs **3**, a 0.4 ppm downfield shift can be seen between the Δ^{23} and its corresponding 23,24-dihydro derivative, and the same is true in the last pair, **5** vs **9**. Concerning **4**, the lowfield value 63.8 is characteristic of a 20-one function and therefore related to the C-17.

The four *methylenic carbons* C-1, 7, 12 and 15 present in cucurbitacin D (**1**) were differentiated by comparing the data of this compound with those of **2**, **4** and **5**. Going from the α-ketol **1** to the diosphenol **5**, the signal due to the C-1 was easily detected. Following acetylation of **1**, the 2-acetoxy group induced the δ of C-1 to move from 36.0 to 32.0 and the 16-acetate resulted in an upfield shift of 2.5 ppm for C-15. Examination of the values found for cucurbitacin D diacetate (**2**) and the corresponding hexanor derivative **4**, indicates that in the latter, only the methylenic C-12 is influenced by the presence of a 20-ketone, following the loss of the side chain. Finally, it should be noticed that the methylenic C-7 in all compounds is under the influence of a strong γ-effect due to

Table 2. ^1H NMR of selected cucurbitacins

Protons	1	2	3	4	5	6	7	8	9
1	-	-	-	β:1.54 (dd 13,6)	5.97 (d 3)	5.90 (d 3)	6.35 (d 3)	6.34 (d 3)	5.96 (d 3)
2	4.44 (dd 12,6)	5.48 (dd 13,6)	4.42 (dd 12,6)	5.48 (dd 13,6)	-	-	-	-	-
6	5.78 (m†10)	5.77 (m†12)	5.79 (m†12)	5.79 (m†10)	5.76 (m†10)	5.77 (m†10)	5.79 (m†10)	5.80 (m†10)	5.77 (m†10)
10	-	-	-	-	3.55 (m†7)	3.51 (m†7)	3.53 (m†7)	3.54 (m†7)	3.51 (m†7)
12-α	3.30 (bd 14)	3.24 (bd 15)	3.25 (bd 15)	3.27 (bd 15)	3.28 (bd 15)	3.20 (bd 15)	3.21 (bd 15)	3.22 (bd 15)	3.23 (bd 15)
12-β	2.70 (d 14)	2.74 (d 15)	2.68 (d 15)	2.53 (d 15)	2.73 (d 15)	2.66 (d 15)	2.78 (d 15)	2.79 (d 15)	2.73 (d 15)
16	4.34 (bt 7)	5.17 (bt 7)	4.31 (bt 7)	5.64 (bt 7)	4.38 (bt 7)	4.39 (bt 7)	5.19 (bt 7)	5.21 (bt 7)	4.35 (bt 7)
17	2.54 (d 7)	2.74 (d 7)	2.61 (d 7)	3.31 (d 7)	2.54 (d 7)	2.49 (d 7)	2.68 (d 7)	2.73 (d 7)	2.61 (d 7)
23	6.62 (d 15)	6.67 (d 15)	-	-	6.62 (d 15)	6.54 (d 15)	6.66 (d 15)	6.41 (d 15)	-
24	7.14 (d 15)	7.13 (d 15)	-	-	7.13 (d 15)	7.03 (d 15)	7.12 (d 15)	7.14 (d 15)	-
Methyls									
18	0.98	1.03	0.97	0.72	1.00	0.97	1.05	1.04	1.00
19	1.40	1.43	1.43	1.33*	1.40	1.42	1.43	1.42	1.44
21	1.36	1.30	1.37	2.17	1.36	1.34	1.30	1.30	1.37
26	1.34*	1.41*	1.22*	-	1.36	1.55	1.41*	1.57*	1.23*
27	1.36*	1.39*	1.25*	-	1.36	1.55	1.39*	1.58*	1.26*
28	1.34	1.31	1.34	1.32*	1.38	1.38	1.33	1.34	1.41
29	1.30	1.28	1.28	1.29	1.26	1.25	1.29	1.29	1.26
30	1.09	1.10	1.08	1.09	1.04	1.02	1.05	1.04	1.04
2-OAc	-	2.14	-	2.13	-	-	2.20	2.20	-
16-OAc	-	1.82	-	2.01	-	-	1.82	1.82	-
25-OAc	-	-	-	-	-	2.01	-	2.02	-

Chemical shifts are in δ units; coupling constants (in Hz) are in parentheses.
*Signals which in any vertical column may be interchanged.
†Value of $W_{\frac{1}{2}}$.

the 19-Me. The high field value of C-7 is thus a characteristic attribute of the cucurbitane skeleton.

The eight *Me carbons* were assigned by direct comparison of the different spectra as shown in Fig. 1, part B. The literature data for oleanane, ursane,[7-9] and dammarane[10] type triterpenes allow the assignment of the signals related to the geminal 28 and 29-Me groups, each having a different δ due to the π orbitals of the C-3 CO and Δ^5 functions. Located in the middle of the cucurbitane skeleton the 18 and 30-Me groups are almost completely independent of any modification taking place in ring A or in the side chain, so that the signals of these Me carbons appear always very close to δ 20. The comparative study of the diacetate **2** with the hexanor derivative **4** led to the assignment of the 19, 21, 26 and 27-Me groups. In the latter (**4**), the 21-Me being α to a ketone, gives rise to a signal at δ 31.3, whereas in the parent structure **2**, it appears at δ 23.6. Again, the chemical shifts resulting from a vicinal acetylation, were useful in differenciating the 26 and 27-Me from the others, but it was not possible to distinguish between the two when comparing **5** (having the 25-OH) with **6** with the 25-OAc.

With such a highly oxygenated molecule, it was expected that the orientation of the side chain in relation to the carbocyclic skeleton and its conformation, might influence the positions of certain signals of the C atoms. Indeed, H-bonding associated with different orientations around the C-17 C-20 bond have been reported for 12β-OH dammaranes[10] and in the withanolides.[11] In the present case the side chain is able to fold in a way which would bring the terminal 25-OH in the vicinity of the 11 ketone. Such a position is favored in a 20R configuration, whereby the 20-OH comes close to the 16α-OH, being probably held by H-bonding. Hence, substituting the 16α-OH or the 25-OH, or both, should influence C atoms even located far away from these positions. Indeed, the 25-OAc group in **6** induces sizable changes in the δ of the C-19, C-11 and C-16, when compared with the values found for a 25-OH **5**, the differences being 1.0, 1.1 and 0.8 respectively. Moreover, the quarternary C-13 and 14 exhibit a difference, although small (0.4), when comparing the same two compounds. The same effect is also reflected by the 26, 27-Me values of their signals which develop different magnetic behaviour. The freedom of rotation around the 24-25 bond being interfered when an H-bond is generated between the 25-OH and the 11-ketone. This can be observed in the differences existing between the signals of the 26 and 27-Me. For example, the difference is 0 in **6**, where the 25-OAc does not allow

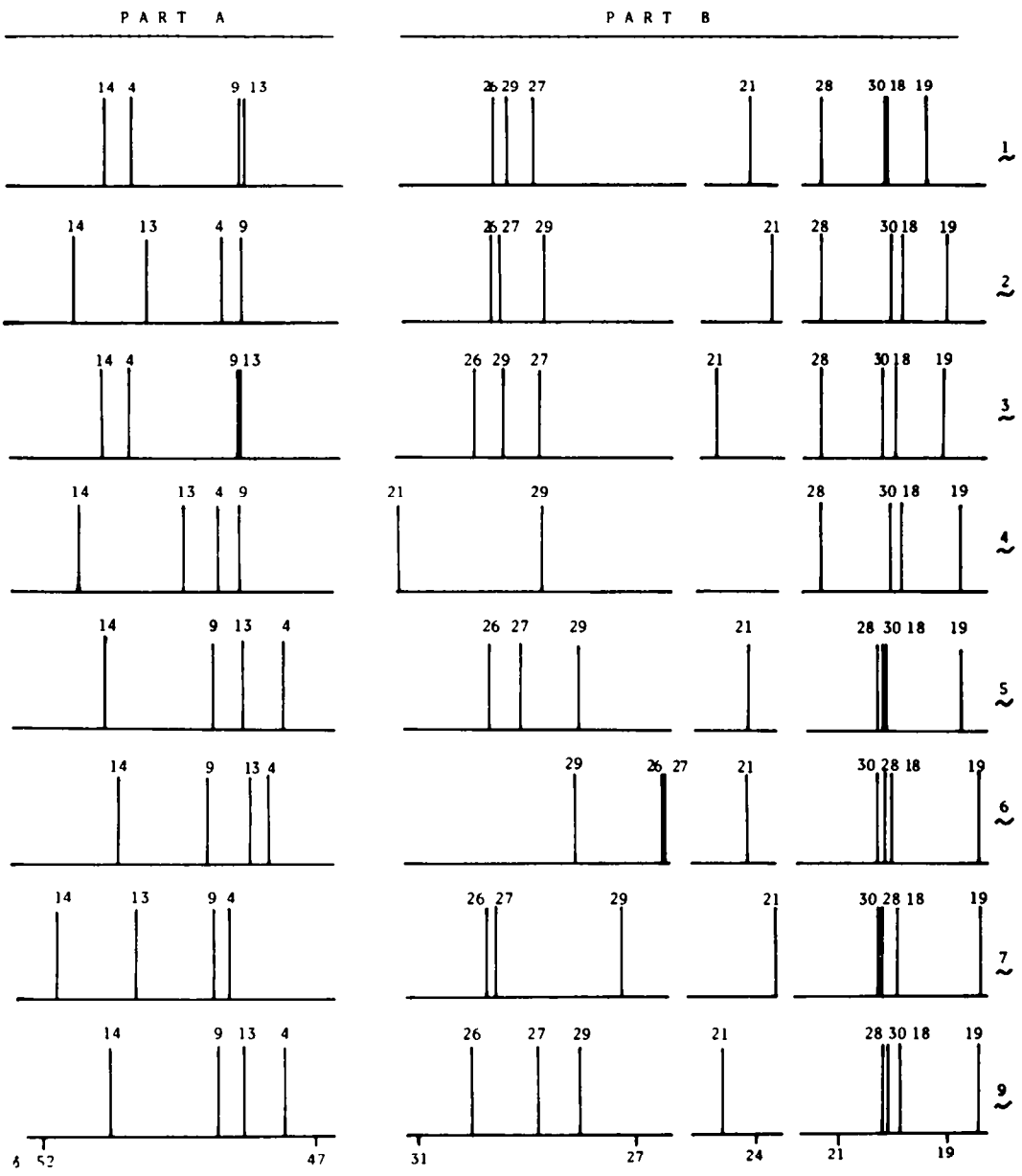

Fig. 1. Comparative ^{13}C NMR location of carbons in the cucurbitacins: (A) quarternary carbons, (B) methyl carbons.

H-bonding (free rotation), and increases to 0.7 and 0.6 in **1** and **5**, where the 25-OH allows bonding to the 11-one (restricted rotation). In the two 23,24-dihydro derivatives **3** and **9**, the folding of the side chain seems facilitated and the bondings of the 25-OH and 20-OH are stronger, restricting even more the rotation of the 26 and 27-Me, the differences in these cases increasing as high as 1.2 ppm in both compounds. In the cases when an OAc group is substituted at C-16, the stability of the folded side chain is lost and the Me groups regain partly their magnetic equivalence and the difference in the values of the signals is reduced to 0.2, 0.1 and 0.3 in **2**, **7** and **8**, respectively.

EXPERIMENTAL

The ^{13}C spectra were recorded on a Bruker WH-90 NMR spectrometer operating at 22.6 MHz in the Fourier transform mode. The ^1H spectra were recorded on a Bruker WH-270 NMR spectrometer operating at 270 MHz in the Fourier transform mode. The δ are derived from $CDCl_3$ soln, except for **6**, for which a small quantity of CD_3OD was added to improve the solubility. All compounds were isolated from *Ecballium elaterium* L. fruit.

Cucurbitacin D (*elatericin A*) **1**, m.p. 151–152° (abs. EtOH), $[\alpha]_D + 52°$ (EtOH).[12,13]

Cucurbitacin D 2,16-*diacetate* **2**, m.p. 184–186° (C_6H_6), $[\alpha]_D - 19°$ ($CHCl_3$).[14]

Cucurbitacin D 23,24-*dihydro* **3**, m.p. 168° (EtOAc), $[\alpha]_D + 83°$ (EtOH).[14]

22-27-*Hexanor-cucurbitacin D* 2,16-*diacetate* **4**, m.p. 202–204° (Et_2O), $[\alpha]_D + 94°$ (MeOH).[15,16]

Cucurbitacin I (*elatericin B*) **5**, m.p. 148–148.5° (aq. MeOH), $[\alpha]_D - 52°$ ($CHCl_3$).[12,13]

Cucurbitacin E (*elaterin*) **6**, m.p. 232–233 (MeOH), $[\alpha]_D - 59°$ ($CHCl_3$).[12]

Cucurbitacin I 2,16-*diacetate* **7**, m.p. 249–250° ($CHCl_3$), $[\alpha]_D$ −78° ($CHCl_3$).[13]

Cucurbitacin E 2,16-*diacetate* **8**, m.p. 124–126° (EtOH).[17]

Cucurbitacin I 23,24-*dihydro* (*cucurbitacin L*) **9**, m.p. 174–176° (Et_2O), $[\alpha]_D$ −47° ($CHCl_3$).[15,18]

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