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Carbon-13 NMR of 5α-Cardenolides

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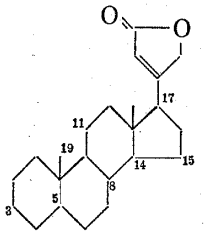
^{13}C-Chemical shifts were obtained on 5α-cardenolides, including uzarigenin and its C-19-, C-11β-, C-15α-hydroxyl-, and Δ^{14}- and $\Delta^{8(14)}$-anhydro-derivatives.

Keywords——C-13 NMR; 5α-cardenolides; anhydrocardenolides; uzarigenin; hydroxy-uzarigenin

Carbon-13 nuclear magnetic resonance (NMR) spectroscopy of 5β-cardenolides[2] was already established as well as those of many other 5α and 5β,14αH-steroids.[3] Concerning 5α, 14βH-steroids, however, few compounds such as 5αH,14β-amino-, azido-, and hydroxy-androstanes were examined.[3a] In this paper, we describe ^{13}C–NMR of 5α-cardenolides, including uzarigenin (I) and its hydroxyl- and anhydro-derivatives. The signal assignment of spectra was based on the usual chemical shift consideration comparing with those of the known 5α- and 5β-steroids,[2,3] and by single-frequency off resonance decoupling (SFORD) technique on some of the samples. The chemical shifts of the cardenolides measured are presented in Table I.



I : 3β,14-dihydroxy-5α-card-20(22)-enolide (uzarigenin)
II : 3β,14-dihydroxy-5α-card-20(22)-enolide 3-O-acetate (uzarigenin acetate)
III : 14-hydroxy-3-oxo-5α-card-20(22)-enolide (uzarigenone)
IV : 3β,11β,14-trihydroxy-5α-card-20(22)-enolide (mallogenin)
V : 3β,14,15α-trihydroxy-5α-card-20(22)-enolide 3-O-acetate
VI : 3β,14,19-trihydroxy-5α-card-20(22)-enolide (coroglaucigenin)
VII : 3β,14-dihydroxy-5α,17βH-card-20(22)-enolide
VIII : 3β-acetoxy-5α-carda-14,20(22)-dienolide
IX : 3β-acetoxy-5α-carda-8(14),20(22)-dienolide
X : 3β-acetoxy-5β-carda-14,20(22)-dienolide
XI : 3β-acetoxy-5β-carda-8(14),20(22)-dienolide
XII : 3β,14-dihydroxy-5β-card-20(22)-enolide 3-O-acetate (digitoxigenin acetate)

As shown in other 5α-steroids,[3] the most upfield peak of all resonances, found at 12.2 ppm in I, is assigned to C 19 methyl carbon by SFORD and by the comparison with those of 19-hydroxy-uzarigenin (VI). Most carbons, arising from A- and B-rings in I are located

1) Location: *Nanakuma 11, Nishi-ku, Fukuoka.*
2) K. Tori, H. Ishii, Z.W. Walkowski, C. Chachaty, M. Sangare, F. Piriou, and G. Lukas, *Tetrahedron Lett.*, **1973**, 1077.
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in lowerfield of those of digitoxigenin by 2—8 ppm, and at approximately the some positions as those of 5α,14αH-steroids, except for C7 and C9, which are observed at 27.9 and 49.9 ppm, respectively and still preserve upper positions by *ca.* 4 ppm than C7 and C9 of 5α,14αH-sterids, due to the steric interaction with C 14—15 bond.[2] The chemical shift of the individual carbon between C6 and C15, except for C12 and C13, is in good agreement with those of 14β-hydroxy-5α-androstane[3a] within a range of ±1.0 ppm.

Introduction of 11β-hydroxyl group in uzarigenin (=mallogenin[4]) (IV) causes long downfield shift of C11 to 66.7 ppm and deshielding of C18 and C19 by 3.5 and 3.3 ppm, respectively, according to δ_1 effect.[3b] C9 and C12, both in β-position from C11, also move to downfield by 4.0 and 8.7 ppm, respectively, C8 being to upfield by 4.5 ppm according to 1,3-diaxial interaction with a methine proton at C8.

15α-Hydroxy-uzarigenin acetate (V) results deshielding at C16 by 11.8 ppm and shielding of C17 by 3.0 ppm, comparing with uzarigenin acetate (II), accompanied by the carbinol shift of C15 from 33.1 to 79.5 ppm.

In 19-hydroxyl derivative of I (=coroglaucigenin[4]) (VI), one carbinol peak is observed at 59.2 ppm instead of the peak at 12 ppm due to C 19 methyl carbon, and the assignment of this resonance is confirmed.

17βH-Uzarigenin (VII) exhibits −8.8 ppm shielding of C 12 as already shown in 17βH-digitoxigenin.[2]

In order to determine the chemical shifts of Δ^{14}- and $\Delta^{8(14)}$-anhydrouzarigenin acetate (VIII and IX, respectively), the corresponding Δ^{14}- and $\Delta^{8(14)}$-anhydrodigitoxigenin acetates

Table I. ^{13}C Chemical Shifts of Uzarigenin and Its Derivatives

Carbon	Compds.											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1	37.4	37.0	38.8	37.5[a]	36.9	33.0[a]	37.5	37.0	37.0	30.7	30.5	30.8
2	32.2	27.3[a]	38.2	32.1	26.9[a]	32.7[a]	32.3	27.8	26.0	24.1[a]	24.8	25.2
3	70.5	73.6	209.7	70.6	73.8	70.7	70.6	73.7	73.6	70.6	70.6	70.6
4	39.0	34.2	44.7	38.7	34.3	39.8	39.1	34.4	34.3	30.7	30.5	30.8
5	44.7	44.4	46.6	45.9	44.1	45.2	44.9	44.4	44.1	37.5	37.8	37.4
6	29.0	28.8	29.4	28.9	29.4	28.8	29.2	28.5	28.8	26.4	26.1	27.0
7	27.9[a]	27.8[a]	27.7[a]	28.2°	27.7[a]	28.1°	27.6	30.1	29.7	25.2	25.6[a]	21.3
8	41.5	41.6	41.9	37.0[a]	42.5	42.4	41.5	35·9[a]	128.6	35.5	129.3	41.8
9	49.9	49.7	49.8	53.9	50.2	50.8	50.2	52.9	49.7	39.8	35.8	35.7[a]
10	35.9	35.8	38.2	36.5	36.0	40.6	36.0	35.5[a]	35.7[a]	35.5	35.8	35.4[a]
11	21.4	21.3	21.8	66.7	21.3	23.4	20.6	22.0	20.0°	22.1	19.8°	21.8
12	39.6	39.6	39.8	48.3	39.6	39.8	30.8	41.2	36.3[a]	41.2	36.9	39.8
13	49.9	49.7	49.8	50.1	49.2	50.2	49.3[a]	48.7	44.1	48.7	43.9	50.1
14	84.5	84.5	84.7	85.8	85.1	84.8	85.2	154.2	139.9	154.2	139.2	84.6
15	32.9	33.1	33.3	33.3	79.5	33.0[a]	31.5	116.5	25.9	116.5	26.1[a]	33.2
16	27.1[a]	27.8[a]	27.4[a]	27.2°	39.6	27.3°	24.8	33.9	27.8	33.9	27.0	27.3
17	51.3	51.4	51.6	52.3	48.4	51.6	48.9[a]	53.8	51.7	52.9	51.9	51.5
18	16.0	16.1	16.2	19.5	17.7	16.3	18.6	18.2	19.4°	18.2	19.2°	16.1
19	12.2	12.1	11.3	15.5	11.2	59.2	12.4	11.9	12.6	23.6[a]	24.2	23.9
20	175.9	175.9	175.4	176.3	175.8	176.2	172.9	171.1[b]	171.5	171.3[b]	171.3[b]	175.9
21	73.6	73.6	73.7	73.8	73.8	73.4	74.1	73.7	73.8	73.8	73.7	73.7
22	117.6	117.7	117.9	117.7	117.1	117.6	116.6	117.1	116.6	116.6	116.8	117.7
23	174.5	174.5	175.4	174.8	175.0	174.6	174.1	171.1[b]	171.9	171.3[b]	171.3[b]	174.4

$-CO\underline{C}H_3$: 21.3±0.1, $-\underline{C}OCH_3$: 170.3±0.2.

a) or ° in vertical column may be reversed.

b) One peak is tentatively shared for C 20 and C 23.

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(X and XI, respectively) were examined. As the results of the removal of C 14-hydroxyl group and the introduction of olefinic linkage between C 14 and C 15, X appears downfield shift of the resonances of C 7 (+3.9 ppm), C 9 (+4.1), C 12 (+1.4), C 16 (+6.6), C 17 (+1.4), C 18 (+2.1), and olefinic C 14 (+69.6) and C 15 (+83.3), comparing with those of digitoxigenin acetate (XII), whereas C 8 (−6.3), C 13 (−1.4), C 20 (−4.6), and C 23 (−3.1) are located in upperfield. XI shows deshielding on the carbons, C 7 (+4.3), C 18 (+3.1), and olefinic C 8 (+87.5) and C 14 (+54.6), while shielding is observed on C 11 (−2.0), C 12 (−2.9), C 13 (−6.2), C 15 (−7.1), C 20 (−4.6), and C 23 (−3.1). Two anhydrouzarigenins were isolated from the reaction mixture according to the same procedure to yield $\Delta^{8(14)}$-anhydrodigitoxigenin.[5] One of them was identified, after acetylation, as VIII by the direct comparison with the sample prepared from II with $SOCl_2$ and pyridine. On the resonance of VIII, similar deshielding as those of X was observed on C 7 (+2.3), C 9 (+3.2), C 12 (+1.6), C 16 (+6.1), C 17 (+2.4), C 18 (+2.1), and olefinic C 14 (+69.7) and C 15 (+83.4), and shielding on C 8 (−5.7), C 13 (−1.0), C 20 (−4.8), and C 23 (−3.4). The shift pattern of the second anhydrocompound (IX) was compared with those of XI. The deshielding of C 7 (+1.9), C 18 (+3.3), and olefinic C 8 (+87.0) and C 14 (+55.4), and shielding of C 11 (−1.3), C 12 (−3.3), C 13 (−5.6), C 15 (−7.2), C 20 (−4.4), and C 23 (−2.6) are quite coincided with those of XI and structure of IX was confirmed as $\Delta^{8(14)}$-anhydrouzarigenin acetate.

Experimental

^{13}C-NMR spectra were recorded on a Hitachi R-22-Ft, at 22.63 MHz. Sample was dissolved in pyridine-D_5 with concentration of 0.2—0.4 mmol/ml and measured at 35° using 8 mm tube with TMS as an internal reference. Average scans were 9000—20000.

Sample——Uzarigenin (I) was obtained on the hydrolysis of odoroside B.[6] Uzarigenone (III) was prepared by CrO_3/pyridine oxidation of I. 15α-Hydroxy-uzarigenin 3-O-acetate (V) was prepared according to the procedure[7] from uzarigenin acetate (II) via Δ^{14}-anhydrouzarigenin acetate (VIII) and its 14,15α-oxide. Mallogenin (IV) and coroglaucigenin (VI)[4] were obtained on the hydrolysis of their rhamnosides, isolated from the seeds of *Mallotus japonica*. 17βH-Uzarigenin (VII) was prepared from I by the isomerization with NaOTs and NaOAc in dimethylformamide.[8]

$\Delta^{8(14)}$-Anhydrouzarigenin Acetate (IX)——I (1.2 g) was dissolved in 20 ml of HCOOH and allowed to stand for 4 days at room temperature.[5] The reaction product was saponified with $KHCO_3$ in 80% MeOH for a week to obtain a mixture of anhydrouzarigenin. The mixture was subjected to silica gel chromatography with benzene–acetone as eluant. The first fraction was crystallized from MeOH to give 260 mg of prisms ($\Delta^{8(14)}$-anhydrouzarigenin), mp 242—247°, $[\alpha]_D^{25}$+13.3° (c=0.23, $CHCl_3$), PMR ($CDCl_3$, δ): 0.68 and 0.80 (3H of each, s, C18 and C19), 4.26 (1H, m, C3), 4.76 (2H, d, J=2 Hz, C21), 5.67 (1H, d, J=2 Hz, C22). Acetate (IX): mp 169—171°, $[\alpha]_D^{25}$+13.3° (c=0.13, MeOH). The second fraction was crystallized from MeOH to give prisms (Δ^{14}-anhydrouzarigenin), mp 275—280°, $[\alpha]_D^{25}$−22.4° (c=0.13, $CHCl_3$), PMR ($CDCl_3$, δ): 0.80 and 0.82 (3H of each, s, C18 and C19), 4.26 (1H, m, C3), 4.47 (2H, d, J=2 Hz, C21), 5.23 (1H, d, J=2 Hz, C15), 5.88 (1H, d, J=2 Hz, C22). Acetate (VIII): mp 183—185°, $[\alpha]_D^{20}$−24.9° (c=1.0, $CHCl_3$). On the comparison with authentic VIII, the both samples were in good agreement.

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