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NMR Signal Assignment of 22-Deoxocucurbitacin D and Cucurbitacin D from *Ecballium elaterium* L. (Cucurbitaceae)

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Summary. ^{1}H and ^{13}C NMR signal assignments derived from 2D NMR experiment based correlations are presented for 22-deoxocucurbitacin D and cucurbitacin D. Both derivatives have been isolated from *Ecballium elaterium* L. (Cucurbitaceae).

Keywords. Natural products; NMR spectroscopy; Structure elucidation; *Ecballium elaterium*; Cucurbitacin.

Introduction

Cucurbitacins are highly oxygenated and structurally divers tetracyclic triterpenes. Their main sources are members of the Cucurbitaceae, but they are also known from other plant families [1]. Cucurbitacins are known for their bitter taste and show a broad range of bioactivities [2]. Although known for several decades, NMR signal assignments presented for even widespread derivatives are often incomplete or are based on increment systems or data set comparisons only [3–5]. Since we recently isolated several cucurbitacin derivatives as reference compounds for analytical purposes, we decided to characterize them by 2D NMR experiment (DQF-COSY, HSQC, HMBC, ROESY) based shift correlation data. The resulting ^{1}H and ^{13}C NMR signal assignments for two of these compounds, namely

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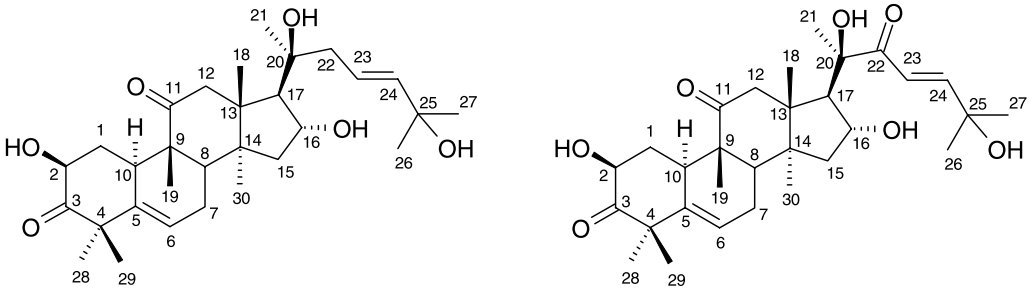

1 **2**

Formula 1

22-deoxocucurbitacin-D (**1**) and cucurbitacin D (**2**) are presented in this contribution (Formula 1).

Results and Discussions

Compound **1** can be considered a rarely found natural product. It has been described from only four sources yet. Beside our account from *E. elaterium* [6] it has been described from two *Bryonia* species [7, 8] and from *Lagenaria siceraria* [9]. Unfortunately, neither the contribution by *Farias et al.* [7] nor the one by *Enslin et al.* [9] provided NMR data for his constituent. The third contribution [8] listed both selected assigned proton shift values and a full set of assigned carbon shift values, but the assignment strategy has not been unveiled. Compound **2** is one of the most abundant cucurbitacin derivatives known. Nevertheless, NMR signal assignments based on shift correlation data have not been provided until very recently [10]. These assignments differ significantly from previous key publications [3–5], which were based on 1D NMR spectra combined with increment rule systems or signal pattern comparisons.

Shift correlation based signal assignment allowed to assign all ^{1}H and ^{13}C NMR shift values of compounds **1** and **2** unequivocally (Table 1 and Fig. 1). The configuration of the substituents at C-4, C-5, C-9, C-10, C-13, C-14, C-17, and C-20 are based on (i) the analysis of the N*O*E network and (ii) biochemical considerations regarding triterpene biosynthesis (especially the stereochemistry at C-5) [11]. Relative stereochemical assignments of methylene group protons at C-1, C-7, C-12, and C-15 were facilitated by analyzing the N*O*E coupling network and the size of the respective proton coupling constants. The resulting signal assignments for 22-deoxocucurbitacin D (**1**) revise the data given by *Panosyan et al.* [8] measured at a proton frequency of 200 MHz. These changes affected not only methyl group assignments, which were not assigned but listed as ''interchangeable'' in the previous contribution but also the oxygen bearing carbons C-2 and C-16. Furthermore, the HMBC based assignments of the carbonyl moieties C-3 and C-11 resulted in a revision of the shift value assignment, too. Further discrepancies were observed for the methylene groups C-15 and C-22. In the case of cucurbitacin D (**2**) the assignment of *Monte et al.* [10] was reproduced.

Table 1. Assigned NMR shift values of 22-deoxocucurbitacin D (**1**) and cucurbitacin D (**2**); given are ^{13}C chemical shifts including their multiplicities and ^{1}H chemical shifts including their multiplicities and coupling constants (J/Hz); measurements were performed at 600 MHz (^{1}H) and 150 MHz (^{13}C) ($CDCl_3$, 300 K)

Compound	**1**		**2**	
Position	δ_C/ppm	δ_H/ppm	δ_C/ppm	δ_H/ppm
1α	36.0 t	2.26 m	36.0	2.31 ddd (3.4, 6.3, 12.7)
1β		1.23 ddd (12.8, 12.8, 12.8)		1.21 ddd (12.7, 12.7, 12.7)
2	71.6 d	4.42 (3.4, 5.5, 12.8)	71.6 d	4.43 ddd (3.4, 6.3, 12.7)
3	213.3 s	–	213.0 s	–
4	50.3 s	–	50.2 s	–
5	140.5 s	–	140.4 s	–
6	120.4 d	5.80 br d (5.5)	120.2 d	5.77 br d (5.8)
7α	23.9 t	2.02 dd (19.5, 5.5)	23.8 t	1.94 ddd (19.8, 5.8, 1.4)
7β		2.41 br dd (19.5, 7.8)		2.39 ddd (19.8, 8.3, 2.9)
8	42.7 d	1.96 d (7.8)	42.3 d	1.96 br d (8.3)
9	48.6 s	–	48.3 s	–
10	33.8 d	2.72 br d (12.8)	33.7 d	2.76 br d (12.7)
11	212.6 s	–	212.2 s	–
12α	48.4 t	3.15 d (14.1)	48.6 t	3.29 d (14.5)
12β		2.48 d (14.1)		2.69 d (14.5)
13	49.8 s	–	50.8 s	–
14	48.0 s	–	48.3 s	–
15α	44.5 t	1.54 d (13.7)	45.5 t	1.35 dd (13.6, 3.9)
15β		1.86 dd (13.7, 7.7)		1.84 dd (13.6, 8.7)
16	72.8 d	4.63 t (7.7)	71.5 d	4.33 br m
17	60.8 d	2.34 br d (7.7)	57.3 d	2.54 d (6.8)
18	19.9 q	0.85	20.0 q	0.97 s
19	20.1 q	1.06	20.1 q	1.07 s
20	75.4 s	–	78.1 s	–
21	25.8 q	1.33	23.9 q	1.39 s
22	46.1 t	2.35 dd (13.7, 6.0) 2.27 dd (13.7, 6.8)	202.5 s	–
23	120.7 d	5.74 m	118.9 d	6.62 d (15.0)
24	144.0 d	5.74 m	155.9 d	7.12 d (15.0)
25	70.8 s	–	71.1 s	–
26	30.0 q	1.34	29.5 q	1.35 s
27	30.0 q	1.34	29.3 q	1.35 s
28	29.4 q	1.27	28.8 q	1.28 s
29	21.2 q	1.34	21.2 q	1.33 s
30	18.8 q	1.38	19.2 q	1.34 s

Experimental

Raw extracts, enriched fractions, and isolated substances were characterized by a HPLC-DAD-MS method based on a method developed previously [12]. Briefly, a HP 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (detection at 200, 230, and 267 nm) and operating at room temperature was used. A Zorbax SB-C18 column

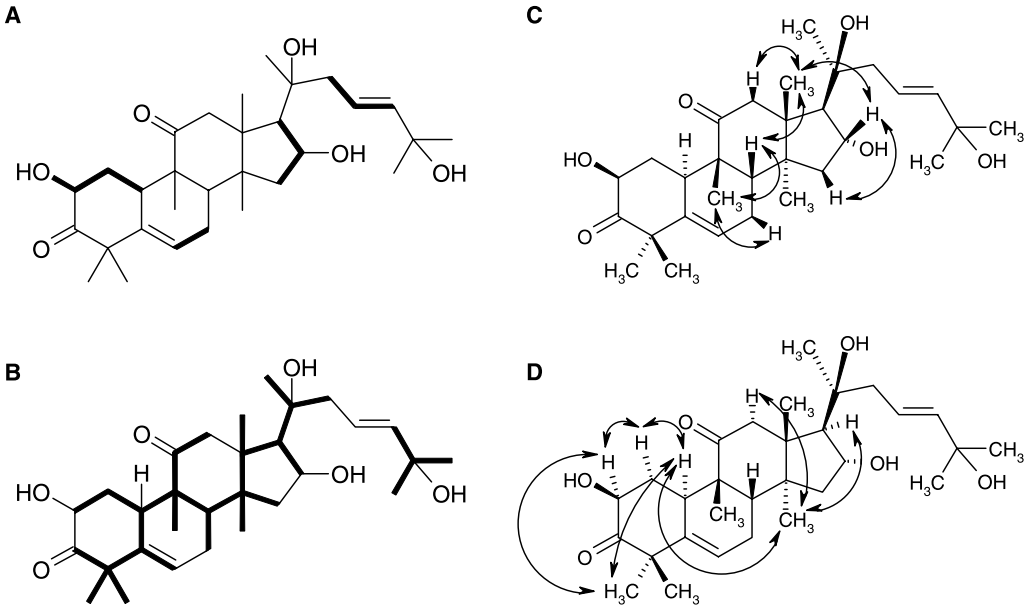

Fig. 1. Formula sketches with 2D NMR experiment derived signal correlations used for the signal assignment of 22-deoxocucurbitacin D (**1**); A: DQF-COSY derived bond connectivities (bold lines); B: HMBC derived bond connectivities (bold lines); C: ROESY derived homonuclear through space correlations for β-oriented substituents (arrows); D: ROESY derived homonuclear through space correlations for α oriented substituents (arrows)

(150 × 4.6 mm, 3.5 µm particle size) (Agilent Technologies, Waldbronn, Germany) was used as stationary phase. A water (solvent A)/acetonitrile (solvent B) gradient (0 min 80% A, 22 min 60% A, 40 min 20% A) served as mobile phase. HPLC-MS experiments were facilitated by coupling of the HPLC system described above to a Bruker Esqire 3000^{plus} mass spectrometer (ion source: ESI, negative mode, spray voltage: 4500 V, nebulizer gas: N_2, 40 psi, dry gas: N_2, 10 dm^3/min, 350°C). The HPLC setup was equal to the HPLC-DAD method, only solvent A was replaced by 0.15% acetic acid. NMR experiments were performed on a Varian Unity Inova 600 in $CDCl_3$ solution at 300 K. All signals were unambiguously assigned by using 2D correlation data (DQF-COSY, HSQC, HMBC, ROESY). Further details on experimental parameters are summarized in *Seebacher et al.* [13].

Isolation

A CH_2Cl_2 extract obtained from *Ecballium elaterium* L. fruit juice (750 cm^3) was subjected to Sephadex LH-20 chromatography in methanol and subsequent counter-current chromatography (HSCCC). Compounds **1** and **2**, co-eluting in the analytical HPLC, were separated by HSCCC (CH_2Cl_2:*Met*OH:H_2O = 50:50:30, lower phase as stationary phase, flow rate 1.5 cm^3/min, coil volume 80 cm^3) yielding 6 mg of pure **1** (elution volume 200–240 cm^3) and 37 mg **2** (elution volume 240–280 cm^3, purity >95%).

2β,16α,20,25-Tetrahydroxy-9-methyl-19-nor-9β,10α-lanosta-5,23-diene-3,11,22-trione (22-deoxocucurbitacin D, **1**)

NMR shift values match with Ref. [8]. Detailed ^1H and ^{13}C NMR assignments are given in Table 1. HMBC contacts (C → H): 2 → 1; 3 → 28, 29; 4 → 6, 28, 29; 5 → 10, 28, 29; 8 → 19, 30; 9 → 19; 10 → 1, 6, 8, 19; 11 → 12, 19; 13 → 12, 18, 30; 14 → 30; 15 → 30; 17 → 18, 21; 18 → 12; 20 → 21, 16; 22 → 21, 23, 24; 24 → 26, 27; 25 → 23, 24, 26, 27; 26 → 27; 27 → 26; 28 → 29; 29 → 28.

2β,16α,20,25-Tetrahydroxy-9-methyl-19-nor-9β,10α-lanosta-5,23-diene-3,11-dione
(cucurbitacin D, **2**)
Chromatographic retention time and HPLC-DAD-MS data were identical to Ref. [12]. NMR shift values match with data in Refs. [3, 5, 10]. ^{1}H and ^{13}C NMR assignments are given in Table 1. HMBC contacts (C → H): 1 → 2; 2 → 1; 3 → 1, 28, 29; 4 → 6, 28, 29; 5 → 7, 28, 29; 6 → 7; 7 → 6, 8; 8 → 6, 19, 30; 9 → 7, 8, 19; 10 → 1, 6, 8, 19; 11 → 19; 12 → 18; 13 → 17, 18, 30; 14 → 12, 18, 30; 15 → 30; 16 → 17; 17 → 12, 18, 21; 18 → 17; 20 → 16, 21; 22 → 17, 21, 23, 24; 24 → 26, 27; 25 → 23, 24, 26, 27; 26 → 27; 27 → 26, 28 → 29; 29 → 28; 30 → 8, 15.

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