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Bryonia ISOPRENES. II. CUCURBITACIN L AND BRYOAMARIDE FROM *Bryonia melanocarpa*

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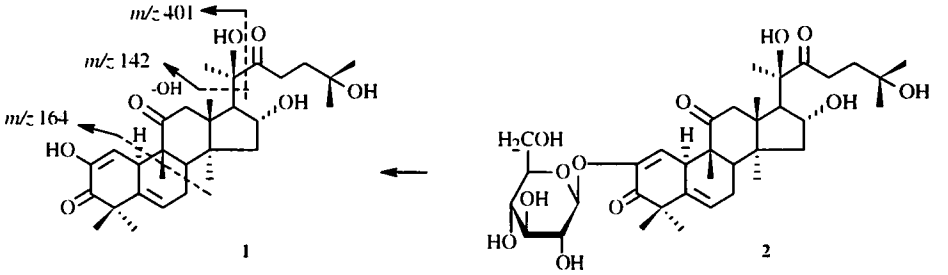
Cucurbitacin L and bryoamaride, the predominant components of the bitter principle of Bryonia melanocarpa *Nab. (Cucurbitaceae), are isolated from the roots of the plant.*

Key words: cucurbitacins, cucurbitacin L, bryoamaride, *Bryonia*, Cucurbitaceae.

We have previously isolated from the roots of the Steppe black-fruited *Bryonia melanocarpa* Nab. (Cucurbitaceae) eight compounds consisting of pentacyclic triterpenes and sterols [1]. In continuation of these studies, we isolated the two principal components of the bitter principle of this plant. These compounds were identified as cucurbitacin L (**1**) and bryoamaride (**2**) and are studied in the present article.

Both compounds have a bitter taste, which suggests that they are methylsteroids of the cucurbitane [19(10→9)-*abeo*-10α-lanostane] series [2]. Other species of this genus have previously also yielded cucurbitacins [3, 4]. The ^1H and ^{13}C NMR spectra of **1** and **2** (see Experimental and Table 1) contain signals of eight tertiary methyl groups and the corresponding C atoms and confirm the correctness of the assignments. The yellow color of **1** and **2** on TLC upon visualization with vanillin—H_3PO_4 indicates that these compounds are 23,24-dihydrocucurbitacins [5].



1 **2**

Cucurbitacin **1** has genin nature; **2**, glycosidic. GLC [6] demonstrated that **2** contains one molecule of D-glucose. The ^1H and ^{13}C NMR are also consistent with this. Signals of one D-glucose are present.

The mass spectrum of **1** lacks a peak for the molecular ion. However, peaks of overlapping ions with *m/z* 401 and 142 are observed. The ion with *m/z* 401 arises from cleavage of the C-20—C-22 bond; with *m/z* 142, dissociation of the C-17—C-20 bond and loss of a hydroxyl. Therefore, it represents a side chain. This means that **1** has a molecular weight of 516 amu. The ion with *m/z* 498 forms after elimination of one water molecule from **1**. The mass spectrometric fragmentation of the latter by electron impact occurs analogously to that of tetrahydrocucurbitacin I [5] and indicates that the side chains are identical.

The structural differences of **1** and tetrahydrocucurbitacin I include an additional double bond located in the polycyclic part of the former. The strong peak with *m/z* 164 obviously results from retrodiene decomposition although the peak corresponding to the second fragment is not observed. The appearance of an ion with *m/z* 164 indicates that the double bond is situated in ring *A* between C-1 and C-2 and forms a diosphenol group. This is consistent with the positive reaction of **1** with iron(III) chloride on TLC and the 1H-doublet for the olefinic proton (H-1) at 6.16 ppm with spin—spin coupling constant (SSCC) $^3J = 3$ Hz in the ^1H NMR spectrum.

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TABLE 1. Chemical Shifts of C Atoms of Cucurbitacin L (**1**) and Bryoamaride (**2**)

C atom	Compound		
	1*[8]	2	2*[9]
1	115.0	123.79	122.69
2	144.7	146.86	146.42
3	198.7	197.02	199.18
4	47.6	48.68	48.59
5	136.9	137.06	136.64
6	120.7	120.90	121.74
7	23.6	23.90	24.05
8	41.6	41.86	42.24
9	48.8	49.67	49.66
10	34.7	35.61	35.61
11	213.00	213.92	216.60
12	48.8	49.48	49.51
13	48.3	49.31	49.66
14	50.8	50.92	51.05
15	45.6	46.52	46.10
16	71.0	70.77	70.68
17	57.8	58.96	58.51
18	19.8	20.19	20.24
19	18.3	18.31	18.46
20	79.2	80.11	80.33
21	24.5	25.52	25.11
22	215.4	216.04	217.13
23	30.9	32.74	32.62
24	37.0	38.46	37.57
25	70.3	69.07	70.24
26	28.7	29.80	29.02
27	29.9	30.07	29.11
28	20.2	20.79	20.55
29	27.9	27.54	27.97
30	20.1	20.36	20.31
		β-*D*-Glc*p*	
1′		100.67	100.22
2′		74.42	73.43
3′		78.67	76.53
4′		70.31	69.85
5′		78.43	77.14
6′		62.05	61.31

*Spectra obtained in $CDCl_3$ [8] and CD_3OD [9] are taken from the cited work.

The structural data presented above characterize cucurbitacin L. The shifts in the ^1H NMR of **1**, taking into account the difference in the chemical shifts of HMDS and TMS in Py-D_5, agree well with those of cucurbitacin L [7]. Thus, we conclude that **1** is cucurbitacin L.

The chemical shifts and SSCC of the monosaccharide in the ^1H NMR spectrum of **2** are consistent with the β-D-glucopyranoside structure for the carbohydrate part of the glycoside. Signals of the olefinic protons H-1 and H-6 are located at 6.29 and 5.57 ppm, respectively, in the spectrum of **2**. Therefore, **2** also contains double bonds at C-1(2) and C-5(6). In fact, enzymatic hydrolysis of **2** produced a genin that corresponded chromatographically with cucurbitacin L. However, the studied glycoside does not give a color reaction with Fe(III) chloride on TLC. Therefore, it seems that the hydroxyl group of the diosephenol function is glycosylated. This is also indicated by the chemical shift of the anomeric C atom of the β-D-glucopyranose (100.67 ppm) and the weak-field shift of signals for C-1 and C-2 compared with those of cucurbitacin L [8, 9].

Thus, **2** is the 2-O-β-D-glucopyranoside of cucurbitacin L. It is known as bryoamaride, which was isolated previously from *Bryonia dioica* Jacq. [4].

EXPERIMENTAL

General Comments [10]. The following solvent systems were used: $CHCl_3$—CH_3OH (15:1) (1), benzene—ethylacetate (1:1) (2), $CHCl_3$—CH_3OH (10:1) (3), $CHCl_3$—CH_3OH—H_2O (70:12:1) (4).

Cucurbitacin was detected by TLC using 25% phosphotungstic acid in ethanol or vanillin—H_3PO_4 with subsequent heating for 3-5 min at 100-110° C.

Samples for diosephenol analysis were sprayed on TLC plates with 5% iron(III) chloride in 50% methanol.

^1H and ^{13}C NMR spectra were recorded on Bruker AM-400, Bruker AC-200, and Tesla BS 567 A instruments in Py-D_5. ^{13}C NMR spectra were obtained with full saturation of C–H coupling and *J*-modulation.

Isolation and Separation of *Bryonia melanocarpa* Isoprenes [1]. The fractions collected between bryonolic acid and glycoside sterols were repeatedly chromatographed on a column using systems 1 and 2 to give one of the principal components, cucurbitacin L. Fractions eluted by system 3 contained the other principal component, bryoamaride, after separation of sterol glycosides. Both compounds are amorphous yellow powders with a bitter taste.

Cucurbitacin L (1). $C_{30}H_{44}O_7$. Mass spectrum, *m/z* (%): M^+ - H_2O 498 (35.3), 480 (97.1), 401 (58.8), 341 (38.2), 219 (26.5), 164 (100), 142 (100).

PMR spectrum (100 MHz, C_5D_5N, 0 = HMDS, δ, ppm, *J*, Hz): 1.00, 1.08; 1.22; 1.24; 1.24; 1.33; 1.43; 1.47 (8×CH_3, s); 2.73 (H-12*a*, d, ^2J = 15 Hz); 2.80 (H-17, d, ^3J = 7 Hz); 3.00-3.40 (H-12*b*, 2H-23); 3.62 (H-10, br. s), 4.76 (H-16, t, ^3J = 7 Hz), 5.53 (H-6, br. s), 6.16 (H-1, d, ^3J = 3 Hz).

Bryoamaride (2). $C_{36}H_{54}O_{12}$. GLC [6] showed that **2** contains one molecule of D-glucose.

PMR spectrum (400 MHz, C_5D_5N, 0 = TMS, δ, ppm, *J*, Hz): 0.89; 1.04; 1.15; 1.25; 1.27; 1.40; 1.51 (8×CH_3, s); 1.59 (H-15β, ^2J = 12.9 Hz); 2.76 (H-12α, d, ^2J = 14.5 Hz); 2.83 (H-17, d, ^3J = 7.1 Hz); 3.10 (H-23*a*, m); 3.26 (H-12*b*, d, ^2J = 15.4 Hz); 3.30 (H-23 *b*, m); 3.60 (H-10, br. s); 3.84 (H-5′, m); 4.00 (H-2′, t, ^3J = 8 Hz); 4.13 (H-4′, t, ^3J = 8.9 Hz); 4.26 (H-3′, t, ^3J = 9.3 Hz); 4.40 (H-6′*a*, dd, ^2J = 12, ^3J = 3.2 Hz); 4.48 (H-6′*b*, dd, ^2J = 12, ^3J = 2.2 Hz); 4.79 (H-16, t, ^3J = 3.2 Hz); 5.30 (H-1′, d, ^3J = 7.8 Hz); 5.57 (H-6, br. s), 6.29 (H-1, d, ^3J = 2.2 Hz). For ^{13}C NMR spectrum, see Table 1.

Enzymatic Hydrolysis of Bryoamaride (2). Glycoside **2** (50 mg) in water (25 ml) was treated with lyophilized gastric juices of the grape snail. The solution was treated with two drops of benzene and incubated at 35 C for 10 days. TLC of the reaction mixture in systems 1, 3, and 4 detected cucurbitacin L (**1**), identical with an authentic sample.

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