Journal of Natural Products
Vol. 53, No. 6, pp. 1600-1605, Nov-Dec 1990

ISOLATION AND ^{13}C-NMR ASSIGNMENTS OF CUCURBITACINS FROM *CAYAPONIA ANGUSTILOBA*, *CAYAPONIA RACEMOSA*, AND *GURANIA SUBUMBELLATA*

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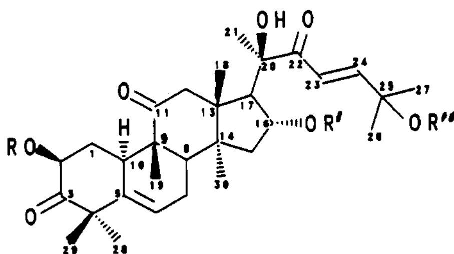
ABSTRACT.—Cucurbitacins B, D, and F and dihydrocucurbitacins D and F have been identified as constituents of *Cayaponia angustiloba*, *Cayaponia racemosa*, and *Gurania subumbellata*; the two *Cayaponia* species also contain a glucoside of cucurbitacin B, arvenin I, not found in *Gurania subumbellata*. Two-dimensional nmr spectroscopy has been used to assign the ^{1}H and ^{13}C spectra of cucurbitacin B, and these assignments have been used as a benchmark to assign the spectra of the other compounds isolated.

The cucurbitacins are of interest biologically because of the wide range of activity they exhibit in plants and animals, and chemically because of their structural complexity as oxidatively modified triterpenes having the 19(10→9β)abeo-10α-lanostane (cucurbitane) skeleton; some occur naturally as glycosides. They are characteristically (but not exclusively) constituents of members of the Cucurbitaceae, which has been divided into two groups of genera: those in which the β-glycosidase elaterase is present and from which only aglycones are isolated, and those in which the enzyme is absent and from which glycosides may be isolated (1).

We have examined three members of the Cucurbitaceae found in Guyana; fruits of *Cayaponia angustiloba* Cogn. and *Cayaponia racemosa* Cogn. and roots of *Gurania subumbellata* (Miq.) Cogn. were extracted. Cucurbitacins B, D, and F, and dihydrocucurbitacins D and F were identified as constituents of all three species; the two *Cayaponia* species also afforded a glucoside of cucurbitacin B, arvenin I, not found in the *G. subumbellata* roots.

Vande Velde and Lavie (2) have listed ^{13}C spectral assignments for a representative selection of cucurbitacins, and ^{13}C spectroscopy has been used in a number of structural studies of cucurbitacins (3–5). A compilation of ^{1}H-nmr data (for pyridine-d_5 solutions) is also available (6). The assignment of ^{13}C chemical shifts to cucurbitacins using empirical rules is difficult because of the closeness of the chemical shifts within several groups of carbons at structurally important centers. The importance of these assignments for structural studies led us to use 2D nmr spectroscopy to place them on firmer ground by establishing ^{1}H-^{13}C connectivities through two or three



1 R=R′=H, R″=Ac
2 R=R′=R″=Ac
3 R=R′=H, R″=Ac, 23,24-dihydro
4 R=R′=Ac, R″=H
5 R=R′=Ac, R″=H, 23,24-dihydro
6 R=R′=R″=H, 23,24-dihydro
8 R=Glc, R′=H, R″=Ac
9 R=GlcAc$_4$, R′=R″=Ac

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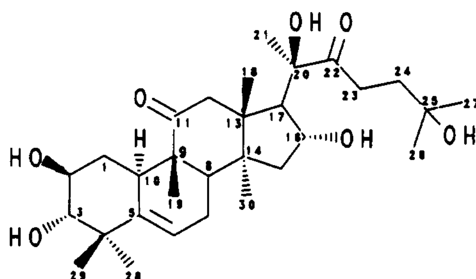

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TABLE 1. Assigned Chemical Shifts and Connectivities for Cucurbitacin B [**1**].

Carbon	n_H	δ_C	δ_H	n-Bond Connectivities[a]		
				Me	CH_2	CH
C-1	2	35.98	2.30, 1.23	(1.08)[b]	—	2.75, 4.43
C-2	1	71.63	4.43	—	1.23, 2.30	2.75
C-3	0	213.03	—	1.29, 1.35	2.30	—
C-4	0	50.23	—	1.29, 1.35	—	(5.80)
C-5	0	140.36	—	—	2.41, 2.30	2.75
C-6	1	120.43	5.80	—	2.41	2.75, 1.98
C-7	2	23.85	2.41, 1.99	—	—	5.80, 1.98
C-8	1	42.37	1.98	1.37, 1.08	2.41, 1.87	5.80
C-9	0	48.43	—	1.08	2.69	2.75, 1.98
C-10	1	33.72	2.75	1.08	2.30, 1.23	(5.80), 1.98
C-11	0	212.17	—	1.08	3.24, 2.69	—
C-12	2	48.63	3.24, 2.69	0.98	—	2.51
C-13	0	50.67	—	1.37, 0.98	3.24, 2.69 1.45	2.51
C-14	0	48.09	—	1.37, 0.98	2.69, 2.41 1.87, 1.45	4.36, 1.98
C-15	2	45.31	1.87, 1.45	1.37	—	1.98
C-16	1	71.23	4.36	—	1.87, 1.45	2.51
C-17	1	58.18	2.51	0.98, 1.44	3.24	4.36
C-18	3	19.83	0.98	—	3.24, (2.69)	2.51
C-19	3	20.02	1.08	—	—	2.75, 1.98
C-20	0	78.37	—	1.44	—	4.36, (2.51)
C-21	3	23.92	1.44	—	—	—
C-22	0	202.49	—	—	—	7.06, (6.49)
C-23	1	120.30	6.49	—	—	(7.06)
C-24	1	151.91	7.06	1.57, 1.54	—	(6.49)
C-25	0	79.31	—	1.57, 1.54	—	6.49
C-26[c]	3	26.41	1.54	1.57	—	(7.06)
C-27[c]	3	25.96	1.57	1.54	—	—
C-28[d]	3	29.35	1.29	1.35	—	—
C-29[d]	3	21.25	1.35	1.29	—	—
C-30	3	18.87	1.37	—	1.87, 1.44	1.98
OCOMe	0	170.23	—	2.02	—	—
O_2CCH_3	3	21.95	2.02	—	—	—

[a]Chemical shift of protons showing n-bond connectivities to the ^{13}C; $n = 2$ or 3 unless otherwise indicated; cross peaks for entries in parentheses are very weak.
[b]Four-bond connectivity.
[c,d]Pairs of methyl groups not differentiated by connectivity experiment.

bonds by techniques we have described previously (7).

Cucurbitacin B [**1**] was readily isolated and identified, and a sufficient amount of homogeneous material was obtained for detailed nmr study. We established the one-bond ^{13}C-^{1}H chemical shift correlation for **1**, and then, with the methodology we have used previously for spectral assignments to triterpenoids (7), we used *n*-bond ($n = 2$ or 3) ^{13}C-^{1}H shift correlations to give a total assignment of its ^{1}H and ^{13}C spectra. Table 1 shows the ^{13}C chemical shifts assigned, hydrogens directly connected to each carbon (one-bond shift correlation), and hydrogens connected through two or three bonds to each carbon (*n*-bond shift correlation). It is apparent that a considerable number of the chemical shifts were misassigned previously. With cucurbitacin B as a benchmark it is possible to make assignments to closely related derivatives such as the 2,16-diacetate **2** (8) and the 23,24-dihydro derivative **3** (9) prepared from cucurbitacin B, but even here there are sites where chemical shift differences are too small for assignments to be made with confidence, and the degree of uncertainty increases as structural differences increase. Similar uncertainty is present in assignments we have made to other cucurbitacins. Completely definitive assignments would require a better supply of homogeneous material for the 2D nmr experiments.

Cucurbitacin D and 23,24-dihydrocucurbitacin D were isolated as a 2:1 mixture (based on the ^{1}H-nmr spectrum) that could not be separated by chromatography. An initial attempt to acetylate the mixture with Ac_2O in the presence of 4-(dimethylamino)pyridine (DMAP) provided three compounds that were separated by chromatography. These were identified as cucurbitacin D 2,16-diacetate [**4**], 2,16,25-triacetate (identical with cucurbitacin B 2,16-diacetate [**2**]), and dihydrocucurbitacin D 2,16-diacetate [**5**]. Prolonged acetylation in the presence of DMAP converted all of the cucurbitacin D to its triacetate, but dihydrocucurbitacin D was not acetylated beyond the diacetate level. Although the 25-OH group is difficult to acylate, DMAP is sufficient to catalyze reaction at this site in cucurbitacin D where the OH can not form a hydrogen bond to the C-22 carbonyl. Even these conditions, however, fail to acetylate the hydrogen-bonded 25-OH of dihydrocucurbitacin D. Catalytic hydrogenation of the original mixture of cucurbitacin D and dihydrocucurbitacin D converted it entirely into dihydrocucurbitacin D [**6**].

Cucurbitacin F and 23,24-dihydrocucurbitacin F were isolated as a 4:1 mixture (^{1}H-nmr spectrum) that could not be separated by chromatography. Catalytic hydrogenation converted the mixture entirely into dihydrocucurbitacin F [**7**]. The identification of this product is based on a comparison of its physical and spectroscopic characteristics with those available for dihydrocucurbitacin F (10) and its stereoisomer cucurbitacin P (dihydrocucurbitacin O) (11).

The glycoside was isolated as a white powder, and treatment with Ac_2O and DMAP converted it to a derivative with five additional acetate groups. The nmr spectra of these compounds indicated that the aglycone was cucurbitacin B and that it carried a glucosidic moiety at the C-2 position. Comparison of the data with those available (3,5) for arvenin I (2-*O*-β-D-glucopyranosylcucurbitacin B) [**8**] and its pentaacetate **9** established that the glycoside was arvenin I. Arvenin I was first identified in *Anagallis arvensis* (Primulaceae) (3), and was later isolated from *Cigarilla mexicana* (Rubiaceae) (5). Its isolation along with free cucurbitacins from members of the Cucurbitaceae appears to be unprecedented.

EXPERIMENTAL

General experimental procedures.—Melting points were determined on a Thomas-

TABLE 2. ^{13}C Chemical Shifts.

Carbon	Compound						
	n_H	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]
C-1	2	36.0	32.0	35.9	32.0	32.0	36.0
C-2	1	71.6	73.5	71.6	73.7	74.1	71.6
C-3	0	213.0	205.6	213.0	205.7	205.7	213.0
C-4	0	50.2	49.8	50.2	49.9	49.9	50.2
C-5	0	140.4	139.6	140.3	139.7	139.7	140.4
C-6	1	120.4	120.4	120.4	120.4	120.4	120.4
C-7	2	23.9	23.5	23.8	23.7	23.7	23.9
C-8	1	42.4	42.0	42.3	42.1	42.0	42.3
C-9	0	48.4	48.3	48.4	48.4	48.3	48.4
C-10	1	33.7	34.2	33.7	34.3	34.2	33.7
C-11	0	212.2	211.9	212.1	211.9	211.9	212.2
C-12	2	48.6	48.5	48.7	48.5	48.5	48.7
C-13	0	50.7	51.2	50.6	51.3	51.2	50.8
C-14	0	48.0	47.9	48.3	48.0	47.9	48.3
C-15	2	45.3	43.0	45.4	43.1	43.2	45.4
C-16	1	71.2	73.2	70.8	73.3	73.3	71.0
C-17	1	58.2	54.0	57.8	54.0	54.0	57.8
C-18	3	19.8	19.7	19.8	19.8	19.7	19.8
C-19	3	20.0	19.9	20.0	20.0	20.0	20.0
C-20	0	78.4	77.6	78.9	77.6	78.7	79.2
C-21	3	23.9	23.7	24.4	23.6	24.3	24.5
C-22	0	202.5	200.8	213.9	201.1	213.5	215.4
C-23	1(2)	120.3	119.1	(30.6)	118.8	(30.7)	(30.8)
C-24	1(2)	151.9	152.6	(34.7)	155.5	(37.1)	(36.9)
C-25	0	79.3	79.1	81.2	71.3	69.9	70.4
C-26[c]	3	26.4	26.6	26.1	29.7	29.6	29.9
C-27[c]	3	26.0	26.3	25.8	28.6	28.6	28.8
C-28[d]	3	29.4	29.2	29.3	29.5	29.4	29.3
C-29[d]	3	21.3	21.2	21.2	21.3	21.3	21.2
C-30	3	18.9	18.6	18.7	18.9	18.9	18.8
OAc signals		170.2	170.3	170.3	—	—	—
		22.0	21.8	22.4	—	—	—
		—	170.1	—	170.1	170.1	—
		—	20.6	—	20.7	21.0	—
		—	169.6	—	169.5	170.1	—
		—	20.6	—	20.0	20.6	—

[a]Assignments made from connectivity experiment.
[b]Assignments made by applying empirical rules to values for **1**.
[c,d]Pairs not differentiated by connectivity experiment; order assigned arbitrarily.

Kofler micro hot stage. Spectrometers used were a Nicolet 5DX FTIR (samples in KBr disks), a Unicam SP1800 for uv spectra (samples in MeOH), a Varian XL 400 for ^{1}H- and ^{13}C-nmr spectra (samples in $CDCl_3$), and a VG70-250S mass spectrometer. A Perkin-Elmer 243B polarimeter was used to measure optical rotations. The one-bond ^{13}C-^{1}H shift-correlation experiment was carried out with the standard Varian HETCOR pulse sequence. The two- and three-bond correlation experiments were carried out with our XCORFE sequence (7,12); two separate experiments were carried out using delays optimized for J_{CH} of 4 Hz and 8 Hz. Spectral parameters were generally similar to those previously reported.

PLANT MATERIAL.—Ripe fruits of *Ca. angustiloba* were collected in February 1985, near the Soesdyke-Linden Highway, Demerara; fruits of *Ca. racemosa* were collected in October 1985, at St. Cuthbert's Mission, Demerara; roots of *G. subumbellata* were collected in December 1986, at Timehri, Demerara. Voucher specimens are deposited at the Herbarium of the University of Guyana and at the Institute of Systematic Botany, University of Utrecht, Netherlands.

EXTRACTION.—Plant material was dried, ground, and extracted with 95% EtOH, and the solvent was removed under reduced pressure. The residue was dissolved in MeOH-H_2O (9:1) and the solution was extracted with hexane. MeOH was removed by evaporation, and the residue was partitioned between H_2O-MeOH (9:1) and EtOAc. The EtOAc fraction provided material for chromatography on SiO_2 with gradient elution (hexane/EtOAc and then EtOAc/MeOH of increasing polarity).

Cucurbitacin B [**1**].—Early fractions provided white cubes, mp 184°, [α]D +79.5° (*c* = 1.5, 95% EtOH). An authentic sample of **1** was available to confirm identity.

2,16-Diacetate of **1**.—Treatment of **1** with Ac_2O, Et_3N, and 4-dimethylaminopyridine (DMAP) gave amorphous **2**, ir 3600, 1738, 1736, 1733 cm^{-1}.

*23,24-Dihydro-***1**.—H_2 and 10% Pd/C converted **1** in MeOH to amorphous **3** no uv absorption >210 nm. ^{13}C nmr data for **1**, **2**, and **3** are listed in Tables 1 and 2.

Cucurbitacin D and dihydrocucurbitacin D [**6**].—The next fractions afforded material that was subjected to tlc purification. A 2:1 mixture of cucurbitacin D and dihydrocucurbitacin D [**6**] was obtained; the ratio was determined from the ^1H-nmr spectrum which included doublets (J = 15.1 Hz) at δ 6.62 and 7.08 (23,24 double bond of cucurbitacin D) and a multiplet at δ 5.70 (C-6 protons of both cucurbitacins).

A CH_2Cl_2 solution of the mixture was treated for 10 h with Ac_2O, Et_3N, and a catalytic amount of DMAP. Preparative tlc then provided cucurbitacin D 2,16,25-triacetate, identical with the 2,16-diacetate of **1**, and amorphous dihydrocucurbitacin D 2,16-diacetate [**5**], no uv absorption >210 nm.

Treatment of the mixture in MeOH with H_2 and 10% Pd/C gave a single isolable product, dihydrocucurbitacin D [**6**], mp 128°, [α]D +97° (*c* = 1.5, $CHCl_3$).

When the original mixture (80 mg) was acetylated with Ac_2O, Et_3N, and a catalytic amount of DMAP for only 2 h, a ternary mixture was obtained. Preparative tlc then provided **2** (35 mg), **5** (18 mg), and cucurbitacin D 2,16-diacetate [**4**] (11 mg), mp 182°, [α]D +86.3° (*c* = 1.5, EtOH). ^{13}C nmr data for **4**, **5**, and **6** are listed in Table 2.

Cucurbitacin F and dihydrocucurbitacin F [**7**].—Later fractions afforded a substance, mp 244–245°, that was a 4:1 mixture of cucurbitacin F and dihydrocucurbitacin F [**7**]; the ratio was determined from the areas of ^1H-nmr signals at δ 6.92 and 7.04 (doublets, 15.3 Hz) and δ 5.72 (multiplet). Treatment of the mixture in MeOH with H_2 and 10% Pd/C gave a single isolable product, dihydrocucurbitacin F [**7**]: mp 158–159° [lit. (9) 155–156°], [α]D +44° (*c* = 0.3 in $CHCl_3$) [lit. (9) +48°], no uv absorption >210 nm; ^{13}C nmr (100 MHz) 18.91 (o), 19.74 (o), 20.13 (o), 23.84 (e), 24.46 (o), 25.31 (o), 26.09 (o), 28.70 (o), 29.20 (e), 29.90 (o), 30.81 (e), 33.64 (o), 36.84 (e), 41.55 (e), 42.63 (o), 45.39 (e), 48.25 (e), 48.39 (e), 48.68 (e), 50.75 (e), 57.67 (o), 68.32 (o), 70.30 (e), 71.04 (o), 78.50 (o), 78.90 (e), 120.82 (o), 137.69 (e), 213.05 (e), 215.45 (e).

Arvenin I [**8**].—The last fractions from the *Cayaponia* species gave a white amorphous material identified as arvenin I (3,5): ir 3500 (br), 1719, 1700, 1690 cm^{-1}; uv 240 nm (ε, 15,200); ^1H nmr (400 MHz; partial listing) 0.92 (s, 3H), 1.03 (s, 3H), 1.25 (s, 3H), 1.26 (s, 3H), 1.32 (s, 3H), 1.41 (s, 3H), 1.50 (s, 3H), 1.54 (s, 3H), 1.99 (s, 3H), 5.73 (m, 1H), 6.49 (d, 15 Hz, 1H), 7.02 (d, 15 Hz, 1H); ^{13}C nmr (100 MHz) 18.83 (o), 19.97 (o), 20.03 (o), 21.28 (o), 21.99 (o), 23.97 (e), 24.12 (o), 25.87 (o), 26.39 (o), 29.25 (o), 34.00 (e), 34.04 (o), 42.31 (o), 45.33 (e), 48.01 (e), 48.42 (e), 48.86 (e), 50.59 (e), 51.31 (e), 58.51 (o), 62.35 (o), 70.02 (o), 71.22 (o), 71.23 (o), 73.73 (o), 76.16 (o), 76.20 (o), 78.23 (e), 79.40 (e), 103.50 (o), 120.34 (o), 120.35 (o), 139.72 (e), 151.98 (o), 170.37 (e), 202.60 (e), 211.29 (e), 213.27 (e).

Treatment of the glycoside in CH_2Cl_2 with Ac_2O, Et_3N, and a catalytic amount of DMAP gave an amorphous product identified as **8** the pentaacetate of arvenin I: ir 3445, 1762, 1748, 1742, 1736, 1731 cm^{-1}; uv 238 nm (ε, 14,300); ^1H nmr (400 MHz) 0.99 (s, 3H), 1.05 (s, 3H), 1.21 (s, 3H), 1.23 (s, 3H), 1.27 (s, 3H), 1.39 (s, 3H), 1.54 (s, 3H), 1.56 (s, 3H), 1.83 (s, 3H), 1.92 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.36 (ddd, 19.9, 7.9, 2.8 Hz, 1H), 2.66 (d, 7.6 Hz, 1H), 2.71 (broad d, 12 Hz, 1H), 2.73 (d, 14.5 Hz, 1H), 3.27 (broad d, 14.5 Hz, 1H), 3.60 (m, 1H), 4.13 (dd, 12.4, 2.5 Hz, 1H), 4.20 (dd, 12.4, 3.8 Hz, 1H), 4.53 (dd, 13.1, 5.7 Hz, 1H), 4.69 (d, 8.0 Hz, 1H), 4.98 (dd, 9.7, 8.0 Hz, 1H), 5.09 ('t', 9.7 Hz, 1H), 5.15 ('t', 7.7 Hz, 1H), 5.21 ('t', 9.5 Hz, 1H), 5.76 (broad d, 6 Hz, 1H), 6.37 (d, 15.7 Hz, 1H), 7.13 (d, 15.7 Hz, 1H); ^{13}C nmr (100 MHz) 18.72 (o), 19.70 (o), 19.76 (o), 20.08 (o), 20.09 (o), 20.61 (o), 20.84 (o), 21.52 (o), 21.78 (o), 21.86 (o), 23.59 (o), 23.75 (e), 26.25 (o), 26.70 (o), 28.72 (o), 33.64 (e), 34.20 (o), 42.12 (o), 48.02 (e), 48.38 (e), 48.44 (e), 48.75 (e), 49.92 (e), 51.26 (e), 54.10 (o), 61.78 (e), 68.51 (o), 71.31 (o), 71.59 (o), 72.61 (o), 73.59 (o), 75.63 (o), 78.24 (e), 79.11 (e), 99.43 (o), 111.23 (o), 120.25 (o), 139.91 (e), 152.59 (o), 169.38 (e), 169.56 (e), 169.80 (e), 170.16 (e), 170.29 (e), 170.89 (e), 200.85 (e), 208.61 (e), 211.49 (e); ms 810 (0.1), 767 (0.2), 700 (0.4), 539 (0.1), 331 (18.5), 169 (40), 111 (100).

ACKNOWLEDGMENTS

We are grateful to Professor David Lavie for a sample of cucurbitacin B. The Centre at the University of Guyana received financial support from the Canadian International Development Agency, and research at the University of Toronto was supported by grants from the Natural Sciences and Engineering Research Council of Canada.

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Received 10 May 1990