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9-METHOXY- AND 7,9-DIMETHOXYTARIACURIPYRONE, NATURAL NITRO-COMPOUNDS WITH A NEW BASIC SKELETON FROM *ARISTOLOCHIA BREVIPES*[1]

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ABSTRACT.—The new natural nitro-compounds, 9-methoxytariacuripyrone [**1**] and 7,9-dimethoxytariacuripyrone [**2**], with the hitherto unknown 5-nitro-2*H*-benzo[h]chromen-2-one skeleton, were isolated from the rhizomes of *Aristolochia brevipes* in addition to compounds **3–6**. The structures were determined by chemical conversions and spectroscopic methods including HMQC and HMBC nmr.

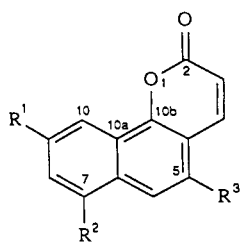
Aristolochia brevipes Benth. (Aristolochiaceae) grows in the eastern parts of the Mexican states of Michoacán and Colima close to the Pacific Ocean. The rhizomes, whose common name is "guaco," are used by the local Tarasc people to treat arthritis and diarrhea, and they are also applied to cure wounds from snake bites (1). From air-dried rhizomes, we isolated six colored compounds, among them **1** and **2**, which are two natural compounds with the new 5-nitro-2*H*-benzo[h]chromen-2-one skeleton.

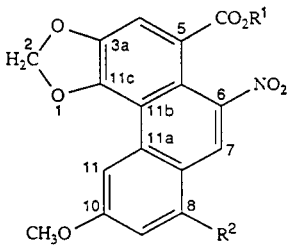
RESULTS AND DISCUSSION

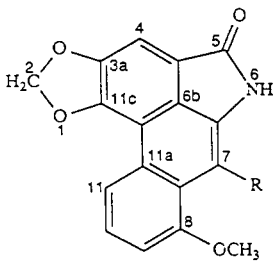
Repeated chromatography of an extract of the rhizomes of *A. brevipes* on Si gel afforded the yellow-colored compounds **1–6**.

In the eims, **1** exhibited a dominant molecular ion at *m/z* 271 ($C_{14}H_9NO_5$) with the base fragment at *m/z* 197 ($C_{13}H_9O_2$). This fragment was explained by loss of NO_2 and CO and indicated a nitro and a carbonyl group. Absorption bands at 1733, 1528, and 1341 cm^{-1} in the ir corroborated these structural elements. ^1H and ^{13}C nmr (Tables 1 and 2) gave evidence for a 1,2,4-trisubstituted benzene unit and an MeO group. Further structural information came from 2D nmr studies, which were done by HMQC (2) and HMBC (3) because of poor solubility. The most important C-H long-range correlations are summarized in Figure 1.

Careful hydrogenation (10% Pd/C) converted **1** to **7**, and this shifted the ^1H-nmr







1 R^1=OMe, R^2=H, R^3=NO_2
2 R^1=R^2=OMe, R^3=NO_2
7 R^1=OMe, R^2=H, R^3=NH_2

3 R^1=R^2=H
4 R^1=Me, R^2=OMe

5 R=H
6 R=OH

[1]Part 53 in the series "Constituents of Tropical Medicinal Plants." For part 52, see R. Torrenegra, J. Pedrozo, J. Robles, R. Waibel, and H. Achenbach, *Phytochemistry*, **31**, 2415 (1992).

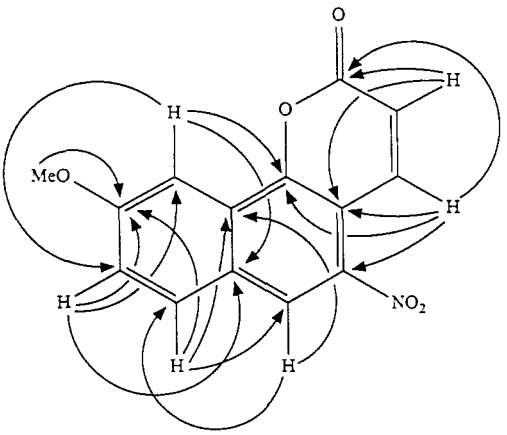


FIGURE 1. Long-range $^{1}H/^{13}C$ couplings observed in **1**.

signals of H-6 as well as H-4 to higher field ($\Delta\delta$ ca. -1.7 and ca. -0.7 ppm), thus indicating the position of the NO_2 group in **1** at C-5. Further evidence for the substitution pattern came from nOe studies on **7** (Figure 2).

The spectroscopic properties of **2** were very similar to those of **1**. Major differences were: (a) the molecular ion and the key fragment $[M-NO_2-CO]^+$ in the ms were shifted 30 mu to higher masses and (b) the signals of a second MeO group appeared in

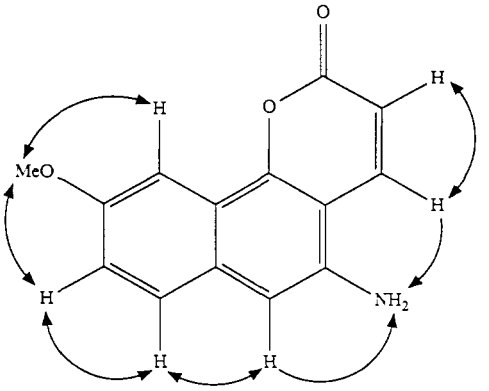


FIGURE 2. Nuclear Overhauser effects observed in **7**.

the nmr's (Tables 1 and 2). The nmr's also established the positions of the MeO groups. Besides **1** and **2**, **6** and the already known natural compounds **3** to **5** were isolated and their structures determined from their spectroscopic properties.

Compounds with nitro groups are rare among natural products, and the best known class of plant-derived nitro-compounds might be the aristolochic acids, which are typical constituents in the Aristolochiaceae (4). They are often accompanied by the biogenetically related aristololactams.

We consider the methyl ester **4** a genuine natural product, since it was also isolated when the extraction and separation procedures were performed with EtOH instead of MeOH and only the acid **3** and no trace of its corresponding methyl ester could be detected in the extracts.

TABLE 1. ^{1}H-nmr (360 MHz, $CDCl_3$) Data for **1**, **2**, and **7**.

Proton	Compound								
	1			**2**			**7**		
H-3	6.68	d	10.0	6.67	d	10.0	6.50	d	10.0
H-4	8.74	d	10.0	8.75	d	10.0	7.93	d	10.0
H-6	8.55	s		8.93	d	0.5	6.86	s	
H-7	7.94	d	9.0	—			7.51	d	9.0
H-8	7.42	dd	9.0, 2.5	6.72	d	2.0	7.18	dd	9.0, 2.5
H-10	7.84	d	2.5	7.40	dd	2.0, 0.5	7.65	d	2.5
7-OMe	—			4.04	s		—		
9-OMe	4.05	s		4.03	s		3.95	s	
NH_2	—			—			3.92	br s	

Aristolochic acid III [**3**] and aristololactam I [**5**] were reported from various *Aristolochia* species (4), and the methyl ester **4** has been described from *Aristolochia kwangsiensis* (5). However, the 7-hydroxylated aristololactam **6** has not been isolated as a plant constituent; only recently **6** was found as a major component when aristolochic acid I was treated with xanthine oxidase (6).

The cyclic skeleton of the hitherto unknown 5-nitro-benzo[h]chromen-2-ones **1** and **2**, for which we suggest the trivial name tariacuripyrone, might originate from a corresponding aristolochic acid: oxidative cleavage of ring A (between C-1 and C-2) brings about **8** and subsequent decarboxylation and oxidative decarboxylation steps directly produce the tariacuripyrones (Figure 3).

This hypothetical biosynthetic pathway is corroborated by the identical substitution pattern observed in **3** and **1** as well as in **4** and **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp's were obtained on a Kofler hot-stage apparatus and are uncorrected. Uv/vis measurements were made in MeOH, and ir in $CHCl_3$. Nmr spectra, if not

TABLE 2. ^{13}C-nmr (90 MHz, $CDCl_3$) Data for **1**, **2**, and **7**.

Carbon	Compound		
	1	**2**	**7**
C-2	159.03	159.11	160.90
C-3	117.62	117.76	114.67
C-4	139.99	140.09	138.66
C-4a	108.63	109.19	107.83
C-5	140.34	139.28	137.41
C-6	122.92	119.27	106.65
C-6a	126.76	119.61	131.45
C-7	131.39	157.97	127.40
C-8	123.60	101.77	122.32
C-9	161.92	163.14	156.49
C-10	101.31	93.59	100.36
C-10a	127.21	127.79	119.05
C-10b	151.20	150.87	151.54
7-OMe	—	56.15[a]	—
9-OMe	56.14	56.22[a]	55.67

[a]Assignments may be interchanged.

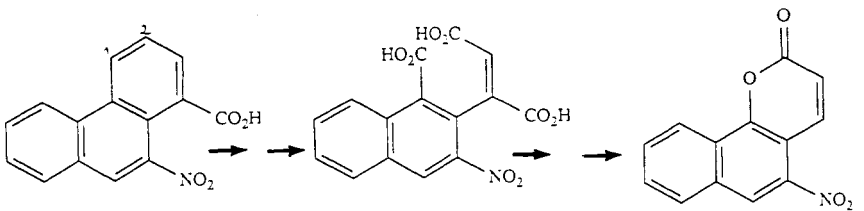


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FIGURE 3. Hypothetical biosynthetic pathway to tariacuripyrones.

otherwise stated, were recorded in $CDCl_3$ with TMS as the internal standard for ^1H nmr at 360 MHz and for ^{13}C nmr at 90 MHz on an AM 360 Bruker instrument. For ^1J-correlation the sequence according to Bax and Subramanian (2) was used, and for long range correlation the sequence according to Bax and Summers (3); best results were achieved by an evolution delay of 60 msec and a relaxation delay of 2 sec. NOe's were measured by the difference method. Ms were run by ei at 70 eV on a Finnigan TSQ 70 instrument, and high resolution measurements on an MAT 311. Only ions with intensities $\geq 5\%$ are given. If not otherwise stated, Si gel for cc was obtained from Macherey-Nagel (No. 81538). Analytical tlc was performed on precoated Si gel plates (Macherey-Nagel, No. 811023) using as solvent petroleum ether-Me_2CO (2:3) with detection by the yellow color and uv fluorescence.

COLLECTION.—The rhizomes of *A. brevipes* were collected in Jiquilpan, Michoacán (México) in September 1988 and botanically identified by Humberto Sánchez V. A voucher specimen is kept at the ITESM herbarium (Reg. No. 8429) in Monterrey.

EXTRACTION AND ISOLATION.—Fresh rhizomes (280 g) were steam-distilled, then air-dried. The dried material was macerated three times with C_6H_6, affording 6 g extract, which was separated by cc on Fractogel TSK 40 using MeOH and then repeated cc on Si gel.

*9-Methoxytariacuripyrone (= 9-methoxy-5-nitro-2*H*-benzo*[h]*chromen-2-one)* [**1**].—Yellow needles (30 mg): mp 228° (from MeOH); tlc R_f 0.65; ir ν max cm^{-1} 1733, 1528, 1341; uv/vis λ max nm (log ϵ) 218 (4.6), 292 (sh), 301 (4.5), 384 (3.9); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 271.0480 (calcd for $C_{14}H_9NO_5$ 271.0481) (100), 243 (8), 225 (12), 213 (12), 198 (9), 197.0603 (calcd for $C_{13}H_9O_2$ 197.0603) (56), 185 (8), 182 (11), 154 (10), 126.0470 (calcd for $C_{10}H_6$ 126.0469) (28).

*7,9-Dimethoxytariacuripyrone (= 7,9-dimethoxy-5-nitro-2*H*-benzo*[h]*chromen-2-one)* [**2**].—Yellow needles (8 mg): mp 224–226° (from MeOH); tlc R_f 0.65; ir ν max cm^{-1} 1737, 1500, 1337; uv/vis λ max nm (log ϵ) 215 (4.3), 280 (sh), 298 (4.3), 404 (3.8); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 301.0591 (calcd for $C_{15}H_{11}NO_6$ 301.0586) (100), 271 (10), 255 (16), 243 (8), 228 (5), 227 (26), 199 (6), 197 (6), 184 (7), 169 (9), 154 (6), 141 (7), 128 (5), 126 (7), 113 (12).

*Aristolochic acid III (=10-methoxy-6-nitrophenanthro-[3,4-*d*]-1,3-dioxole-5-carboxylic acid)* [**3**].—Yellow crystals (3 mg): mp 260–270° (from MeOH); tlc R_f 0.50; ^{13}C nmr (in pyridine-d_5) δ ppm 167.9 (C=O), 161.6 (C-10), 146.9 (C-11c), 146.0 (C-3a), 144.3 (C-6), 132.8 (C-8), 132.2 (C-11a), 127.5 (C-7), 124.3 (C-5), 122.3 (C-7a), 119.7 (C-9), 118.7 (C-5a), 117.8 (C-11b), 112.8 (C-4), 112.5 (C-11), 102.9 (C-2), 51.9 (OCH_3). Ir, uv/vis, ^1H nmr, ms in accordance with published data (7).

*Aristolochic acid IV methyl ester (= 8,10-dimethoxy-6-nitrophenanthro[3,4-*d*]-1,3-dioxole-5-carboxylic acid methyl ester)* [**4**].—Yellow-orange crystals (2 mg): mp 232–234° (from MeOH); tlc R_f 0.60, yellow-orange fluorescent. Spectroscopic properties in accordance with published data (8–11).

Aristololactam I (= 8-methoxybenzo[f]*-1,3-benzodioxolo*[6,5,4-*cd*]*indol-5(*6H*)-one* [**5**].—Yellow needles (16 mg): mp 287–289° (from MeOH); tlc R_f 0.48; light-blue fluorescent. Spectroscopic properties in accordance with published data (11,12).

7-Hydroxy-aristololactam I (= 7-hydroxy-8-methoxybenzo[f]*-1,3-benzodioxolo*[6,5,4-cd]*indol-5(*6H*)-one* [**6**].—Light-yellow fluorescent crystals (3 mg): 290° (dec) (from MeOH); tlc R_f 0.28; uv/vis, ^1H nmr (DMSO-d_6), ms in agreement with published data (6); ^{13}C nmr (pyridine-d_5) δ ppm 168.8 (C=O), 157.4 (C-8), 147.7 (C-3a), 147.4 (C-11c), 134.8 (C-6a), 128.7 (C-11a), 127.9 (C-6b), 126.4 (C-10), 121.7 (C-11), 119.2 (C-4a), 118.4 (C-7a), 109.7 (C-9), 108.0 (C-11b), 106.6 (C-4), 103.2 (C-2), 56.6 (OCH_3), signal of C-7 hidden by solvent signals; eims *m/z* (%) $[M]^+$ 309.0634 (calcd for $C_{17}H_{11}NO_5$ 309.0637) (92), 295 (20), 294 (100), 238 (38), 210 (11), 182 (14), 164 (15), 154 (27), 132 (27).

5-Amino-9-methoxy-2H-benzo[h]*chromen-2-one* [**7**].—Compound **1** (5 mg), suspended in 1 ml MeOH, was hydrogenated over Pd/C (10%) (room temperature, 1 bar, 30 min). Filtration and evaporation afforded **7** as brown-yellow needles (5 mg): mp 242–243° (from MeOH); tlc R_f 0.45; ir ν max cm^{-1} 3354, 3019, 1728, 1604, 1563, 1508; uv/vis λ max nm (log ϵ) 351 (sh), 334 (sh), 306 (3.93), 276 (3.89), 268 (sh), 238 (4.31), 222 (sh); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 241 (100), 226 (61), 198 (39), 170 (18), 141 (10), 115 (15).

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