Notes

Achyrofuran, a New Antihyperglycemic Dibenzofuran from the South American Medicinal Plant *Achyrocline satureioides*

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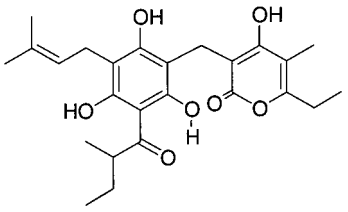
A new prenylated dibenzofuran, achyrofuran (**2**), was isolated from an extract of *Achyrocline satureioides* by bioassay-guided fractionation using the *db*/*db* mouse model for type 2 diabetes. Compound **2** significantly lowered blood glucose levels in this model when administered orally at 20 mg/kg q.d.

Achyrocline satureioides (Lam.) DC. (Asteraceae) was investigated as part of Shaman's ethnobotanical-directed search for novel drugs for the treatment of type 2 diabetes (non-insulin-dependent diabetes mellitus; NIDDM). This species is a widely used medicinal plant in South America, where it is commonly known as "marcela", "macela", and "macela-do-campo",[1] and as such it has received considerable attention by chemists.[2] Among its many reported uses is as a remedy for type 2 diabetes,[1,3,4] but no specific compounds have been ascribed to this activity. Herein we report the isolation and structure elucidation of a new compound, achyrofuran (**2**), which displayed significant antihyperglycemic activity in an in vivo type 2 diabetes mouse model.

Extracts of *A. satureioides* whole plant material were significantly active in our in-house type 2 diabetes model using genetically diabetic *db*/*db* mice.[5] Bioassay-directed fractionation of the aqueous EtOH extract by liquid−liquid partitioning followed by HP-20 chromatography and high-speed countercurrent chromatography (HSCCC) led to a single active fraction. The major component of this fraction was purified by another cycle of HSCCC and identified as 23-methyl-6-*O*-desmethylauricepyrone (**1**), a known compound previously reported from this plant.[6] Compound **1**, however, was not significantly antihyperglycemic. The active material from the first HSCCC run was subjected to LH-20 chromatography, which clearly separated the active material from **1**. HSCCC followed by ODS MPLC led to **2** as the active compound.

HREIMS and NMR data indicated a molecular formula of $C_{32}H_{40}O_8$ for **2**. The NMR data of **2** (Table 1) revealed that it shared several structural features with **1**: a $\Delta^{2,3}$ isoprenyl group and not one, but two α-methylbutyryl functions. The structure of the remaining aliphatic portion, a 2-hydroxy-3-methyl-3-butenyl group, which is a common structural motif of prenylated phloroglucinols, was readily



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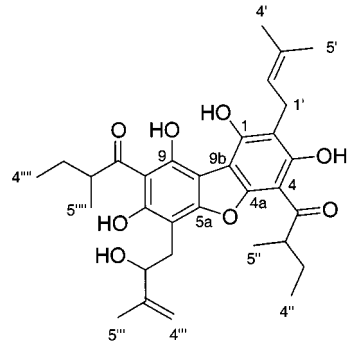

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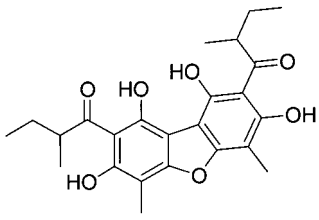

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established from 2D NMR data and comparison to literature values.[7] These subunits accounted for all the carbon-bound hydrogens, 20 of the carbons, and three of eight oxygen atoms. The chemical shifts of the remaining 12 carbon signals were consistent with **2** being comprised of two trisubstituted phloroglucinol units, but the number of double-bond equivalents for its molecular formula and the oxygen count required the formation of a third ring with an oxygen atom common to both of the phloroglucinol units. A dibenzofuran moiety met the prerequisite. The substitution pattern of the dibenzofuran chromophore remained to be established.

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Table 1. NMR Data for **2**

position	^{13}C	^{1}H	HMBC correlations
1	154.6		
OH-1		~10 (1H, br s)	C-1, C-2
2	111.7		
3	163.9		
OH-3		14.0 (1H, s)	C-1, C-2, C-3, C-4, C-1″
4	101.3		
4a	154.1[a]		
5a	158.2[b]		
6	102.3		
7	157.5[b]		
OH-7		~16 (1H, br s)	
8	107.2[c]		
9	155.5[a]		
OH-9		~10 (1H, br s)	
9a	104.7[c]		
9b	105.2[c]		
1′	21.8	3.46 (2H, d, 7.0)	C-1, C-2, C-3, C-2′, C-3′
2′	122.2	5.32 (1H, tm, 7.0, < 1)	C-2, C-1′, C-4′, C-5′
3′	132.2		
4′	25.8	1.71 (3H, d, 1.0)	C-2′, C-3′, C-5′
5′	17.9	1.85 (3H, s)	C-2′, C-3′, C-4′
1″	207.0		
2″	45.4	3.81 (1H, sextet, 6.5)	C-1″, C-3″, C-4″, C-5″
3a″	26.4	1.56 (1H, m)	C-1″, C-2″, C-4″, C-5″
3b″		1.86−1.92 (1H, m)	
4″	11.9	0.94 (3H, t, 7.5)	C-2″, C-3″
5″	17.0	1.26 (3H, d, 6.5)	C-1″, C-2″, C-3″
1a‴	30.3	3.07 (1H, dd, 15.0, 9.0)	C-5a, C-6, C-7, C-2‴, C-3‴
1b‴		3.20 (1H, dd, 15.0, 2.0)	C-5a, C-6, C-7, C-2‴, C-3‴
2‴	78.0	4.53 (1H, m)	C-6, C-1‴, C-3‴, C-4‴, C-5‴
3‴	145.8		
4a‴	111.7	4.97 (1H, br s)	C-2‴, C-3‴, C-5‴
4b‴		5.10 (1H, br s)	C-2‴, C-3‴, C-5‴
5‴	18.2	1.89 (3H, s)	C-2‴, C-3‴, C-4‴
1⁗	213.4		
2⁗	46.3	4.01 (1H, sextet, 6.5)	C-1⁗, C-3⁗, C-4⁗, C-5⁗
3a⁗	27.0	1.49 (1H, m)	C-1⁗, C-2⁗, C-4⁗, C-5⁗
3b⁗		1.86−1.92 (1H, m)	
4⁗	11.9	0.96 (3H, t, 7.5)	C-2⁗, C-3⁗
5⁗	16.3	1.23 (3H, d, 7.0)	C-1⁗, C-2⁗, C-3⁗

[a−c] Values are interchangeable with the same superscript.

From HMBC correlations from H-1′ to C-1, C-2, and C-3 and from H-1‴ to C-5a, C-6, and C-7, it appeared that the isoprenyl and 3-methyl-3-butenyl subunits belonged to different phloroglucinol subunits. Four exchangeable proton signals were observed in the ^{1}H NMR spectrum of **2**. Fortunately, one of these was an extremely sharp singlet resonating at δ 14.0 (OH-3). In addition to strong two- and three-bond correlations in an HMBC experiment to C-2, C-3, and C-4, a cross-peak was also observed from this signal to a carbonyl signal of one α-methylbutyryl groups. It was thus unequivocal that each of the α-methylbutyryl groups belonged to a different phloroglucinol subunit.

Finally, the orientation of the phloroglucinol subunits through the furan ring was determined from DPFGSE-NOE experiments. Irradiation of H-2″generated NOEs at H_2-1‴, H-2‴, and H-4b‴, as well those expected at H_2-3″, H-4″, and H-5″. Only enhancements at H-3⁗, H_2-4⁗, and H-5⁗ were observed when H-2⁗ was irradiated. These results secured the structure of **2**. The stereochemistry at the three stereogenic centers remains to be determined.

When *db*/*db* mice were dosed orally at 20 mg/kg q.d., **2** lowered serum glucose levels below those in the positive control group treated with metformin, a drug used to treat type 2 diabetes in humans, dosed at 250 mg/kg q.d. (Table 2). A slight decrease in body weight and food intake was observed in the group treated with **2**, but the animals appeared healthy during treatment.

The structure of **2** bears some resemblance to the those of the rhodomyrtoxins (e.g., rhodomyrtoxin B, **3**), which were isolated from fruits of the Australian finger cherry *Rhodomyrtus macrocarpa* (Myrtaceae).[8] These compounds received their dubious name as they were believed initially to be responsible for causing sudden and permanent blindness of 27 humans and for poisoning livestock when the tree's unripe fruits were consumed.[9] Subsequently it was shown that one of the rhodomyrtoxins did not cause blindness in several different animal models when administered orally or directly into the eye.[8b] Achyrofuran (**2**) also had no apparent acute toxic effect at the dose level (20 mg/kg) of our tests.

Experimental Section

General Experimental Procedures. UV spectra were measured in MeOH on a Beckman DU 640 spectrophotometer. NMR spectra were acquired on Varian Unity Plus 400 MHz and Bruker DRX-600 spectrometers in $CDCl_3$ which had been passed through basic alumina cartridges just prior to use. A 60 ms Gaussian pulse width with 1K data points truncated at 3% was used for the DPFGSE experiments. Mass spectra were measured on a Kratos MS 50 TC spectrometer. A PC Inc. multilayer-coil separator-extractor equipped with a Rainin HPXL solvent delivery system and an ISCO UA-5 absorbance monitor (254 nm) was used for high-speed countercurrent chromatography (HSCCC). Heptane−CH_2Cl_2−MeCN (5:1.5:3.5) at 4 mL/min was used as the solvent system for HSCCC, with either the lower phase (first cycle) or upper phase (second cycle) as the mobile phase. Column chromatography was carried out using ODS (40 μm) from J. T. Baker, HP-20 from Mitsubishi Chemical Corporation, and Sephadex LH-20.

Plant Material. *Achyrocline satureioides* was collected from the Banda de Chilcay district, in San Martín, Perú, by Dr. Franklin Ayala in May 1997. The plant was authenticated by Mary Merello of Missouri Botanical Garden. Voucher specimens (#8089) have been deposited at the Missouri Botanical Garden.

Bioassay. The type 2 diabetes *db*/*db* mouse model bioassay has been described in detail elsewhere.[5] Each treatment group consisted of eight mice. Metformin was formulated in 0.25% carboxymethyl cellulose (CMC), while test materials were formulated in 10% DMSO, 1% Tween 60, and water. Results are presented as the mean ±SEM, and differences were evaluated by Student's *t*-test or one-way analysis of variance (ANOVA). Differences of $p < 0.05$ were considered statistically significant.

Extraction and Isolation. The air-dried whole plant material of *A. satureioides* (6 kg) was homogenized in 80%

Table 2. Changes in Plasma Glucose Levels, Body Weight, and Food Consumption for Vehicle, Positive Control, and **2** in *db*/*db* Mice

		plasma glucose levels (mg/dL)			body weight, average (g/mouse)		food intake (g/mouse)
treatment	dose (mg/kg), q.d.	0 h	3 h	27 h	0 h	24 h	average
vehicle		368.0 ± 13.4	340.9 ± 15.4	340.8 ± 14.9	38.9 ± 0.4	39.1 ± 0.4	7.1
metformin	250	368.0 ± 7.9	263.2 ± 12.7	232.7 ± 29.0	39.0 ± 0.5	38.9 ± 0.5	5.4
achyrofuran (**2**)	20	367.8 ± 13.0	217.6 ± 19.3	169.0 ± 7.6	37.7 ± 0.5	37.0 ± 0.6	3.9

aqueous EtOH (101 L), filtered, and concentrated (553 g). A 545 g portion of the extract was partitioned between CH_2Cl_2−H_2O (1:1, 32 L), from which was obtained 170 g of lower layer residue. A 135 g portion of this material was dissolved in 90% aqueous EtOH (3 L) and extracted thrice with petroleum ether (2 L for the first, then 1 L each for the second and third extractions). The lower layer residue (77 g) was fractionated over HP-20 (2 L column volume) by eluting with 2.5 column volumes each of 90% MeOH, MeOH, and CH_2Cl_2. Only the CH_2Cl_2 eluent residue (37.4 g) displayed antihyperglycemic activity. Two 2 g portions of the CH_2Cl_2 residue were each fractionated by HSCCC (28 mL/fraction, lower phase as the mobile phase). The pool of fractions 8−10 from both runs (924.1 mg), which were combined due to their similar ^{1}H NMR spectra and TLC profiles (silica gel, CH_2Cl_2−MeOH, 9:1), showed significant antihyperglycemic activity. The major metabolite in these active fractions was purified by another cycle of HSCCC (upper phase as the mobile phase) and found to be the known compound 23-methyl-6-*O*-desmethyl auricepyrone (**1**)[6] (0.038% yield based on whole plant dry weight). Compound **1** was not significantly active in the *db*/*db* mouse model bioassay. Additonal extract was processed up through the first HSCCC step, but rather than subjecting the pool of fractions 8−10 from two runs (1 g) to another cycle of HSCCC at this point, it was fractionated over LH-20 (2.5 × 76.2 cm, 7.5 mL/fraction) by eluting with CH_2Cl_2−MeOH (1:1). Fractions 20−30 (506.5 mg) contained **2** and were pooled. Two 170 mg subsamples of the active material were each now subjected to a second cycle of HSCCC, using the upper phase as the mobile phase. Fractions containing **2** (TLC analysis) were pooled (17 mg). Final purification by ODS MPLC (10 g), eluting with MeCN−0.05 M NH_4OAc (1:1), afforded 3.8 mg of **2** (0.00023% of dried whole plant material).

Achyrofuran (2): UV (MeOH) λ_{max} 224, 240, 280, 288, 372 nm; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 552.2685 (calcd for $C_{32}H_{40}O_8$, 552.2723).

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