Antihyperglycemic Sesquiterpenes from *Psacalium decompositum*

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Psacalium decompositum was investigated for antihyperglycemic compounds using diabetic *ob*/*ob* mice as a model for type 2 diabetes. In vivo bioassay-guided fractionation of an aqueous extract from the roots of *P. decompositum* led to the isolation of two new eremophilanolides, 3-hydroxycacalolide (**1a**) and *epi*-3-hydroxycacalolide (**1b**). A 1:1 mixture of **1a**/**1b** exhibited antihyperglycemic activity when tested at 1.09 mmol/kg in *ob*/*ob* mice. The known furanoeremophilanes, cacalone (**2a**) and epicacalone (**2b**), were also isolated from the aqueous extract and were inactive. The known furanoeremophilane, cacalol (**3**), was isolated from a CH_2Cl_2 extract of *P. decompositum* roots and possessed antihyperglycemic activity. The relative stereochemistry in **1a** and **1b** was assigned 3*R**,5*S** and 3*S**,5*S**, respectively, based on ROESY data, $^3J_{H-H}$ values, and molecular mechanics calculations. Complete ^{13}C and ^1H NMR chemical shifts were assigned for **1a**, **1b**, **2a**, **2b**, and **3**, and several revisions in ^{13}C NMR assignments for **2a** and **3** were made. Results from the conformational analysis of **1a**, **1b**, **2a**, and **2b** indicate that each compound exists in one major conformation in solution with H_3-12 in a pseudoaxial position.

As part of our ethnobotanical approach to discover and develop drugs for the treatment of type 2 diabetes,[1,2] also known as noninsulin-dependent diabetes mellitus, *Psacalium decompositum* (A. Gray) H. Robins and Bret. (family Asteraceae, tribe Senecioneae)[3] was investigated for antihyperglycemic compounds. *P. decompositum* is taken orally in Mexico and the United States, usually in the form of aqueous decoctions, as a remedy for diabetes.[4−8] The aqueous extract of *P. decompositum* roots and the aqueous or methanolic extracts of *P. peltatum* roots were reported to produce hypoglycemic activity;[9−11] however, no chemical investigation regarding this hypoglycemic activity has been carried out. Asteraceae plants of the tribe Senecioneae are known to be rich in pyrrolizidine alkaloids.[12] Pyrrolizidines, with 1,2-unsaturation in the necine ring and esterification at C-9, are metabolized by oxidase enzymes in the liver to a reactive pyrrole that undergoes alkylation in the liver or lung, leading to hepatotoxicity or pulmonary damage.[13] 1,2-Unsaturated pyrrolizidines have been detected by TLC in the aqueous extract of *P. decompositum* and may represent a health hazard when consumed as an herbal medicine.[14] Therefore, we focused our search for antihyperglycemic compounds within the nonalkaloid extracts of *P. decompositum*.

In vivo-guided fractionation of the aqueous extract from the roots of *P. decompositum*, using C57BL-6J *ob*/*ob* mice as a model for type 2 diabetes, led to the isolation of two new eremophilanolides, 3-hydroxycacalolide (**1a**) and *epi*-3-hydroxycacalolide (**1b**). The details of the extraction, isolation, structure elucidation, conformational analysis, and antihyperglycemic activity of these compounds are the subject of this paper.

Results and Discussion

The aqueous extract of the roots of *P. decompositum* exhibited glucose-lowering activity in diabetic *ob*/*ob* mice after a single oral dose at 1000 mg/kg (Table 1). An alkaloid extraction was carried out on the aqueous extract, and fractions containing nonalkaloids, free-base alkaloids, and *N*-oxide alkaloids were generated. The free-base and *N*-oxide fractions tested positive for pyrrolizidine alkaloids by TLC with Ehrlich's reagent.[15] Both nonalkaloid and *N*-oxide alkaloid fractions tested positive in *ob*/*ob* mice with a single dose at 500 mg/kg (Table 1), but no further work was done on the *N*-oxide fraction due to the presence of pyrrolizidine alkaloids. Purification of the nonalkaloid fraction by HPLC yielded **1a**, **1b**, and two known furanoeremophilanes, cacalone (**2a**) and epicacalone (**2b**).

3-Hydroxycacalolide (**1a**) was obtained as an amorphous white solid. The IR spectrum indicated the presence of a β,γ-unsaturated γ-lactone ring (1798 cm^{-1}),[16] hydroxyl group (3374 cm^{-1}), and phenyl ring (1635 cm^{-1}). The molecular formula, $C_{15}H_{18}O_4$, was determined by HREIMS *m*/*z* 262.1216 (M^+, Δ 1.1 mmu of calc), ^{13}C and DEPT NMR. Analysis of the ^{13}C NMR of **1a** (Table 2) indicated the presence of one lactone carbonyl carbon and three tetrasubstituted double bonds; consequently, this required a dihydroxytricyclic structure. The ^1H NMR of **1a** (Table 3) exhibited signals for three distinct methyls, a tertiary methyl at δ 1.69 (s, H_3-10), a tertiary methyl at δ 2.29 (s, H_3-11), and a secondary methyl at δ 1.10 (d, $J = 6.8$ Hz, H_3-12). A COSY spectrum identified two separate proton spin systems; H_2-6 to H_3-12 were part of a $-CH_2-CH-CH_3$ group, and H_2-7 to H_2-8 composed a $-CH_2-CH_2-$ group. Although no coupling between H_2-6 and H_2-7 was clearly observed in the COSY spectra due to overlap of chemical shifts, a HMBC correlation between H-8β and C-6 suggested that the two spin systems were connected at C-6 and C-7. The ^{13}C chemical shifts of C-5 through C-8 were reminiscent of those reported for cacalol (**3**).[17] The rearranged eremophilanolide skeleton in **1a** was further substantiated by long-range C−H correlations provided by HMBC and long-range HETCOR experiments. Long-range correlations between H_3-12 and C-4a and C-5, along with correlations between H_3-11 and C-4a and C-4, established the positions of C-4 (δ 124.8) and C-4a (δ 138.5). HMBC correlations observed between H_3-10 and C-2, C-3, and C-3a, in addition to a correlation observed between H_3-11 and C-3a, provided the basis for assignments of C-2, C-3, and C-3a in the lactone ring. The chemical shift of C-3 (δ

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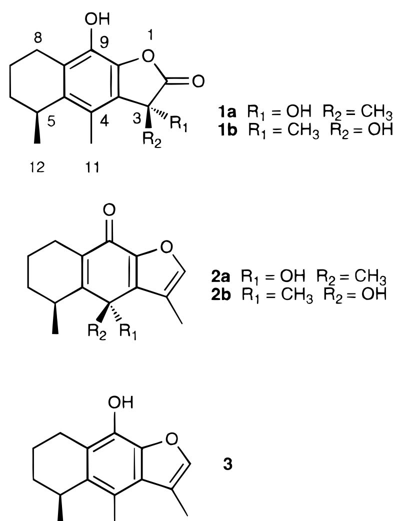
Table 1. Change in Glucose Level, Body Weight, and Food Consumption in *ob*/*ob* Mice Receiving Vehicle, Positive Control, *P. decompositum* Aqueous Extract, Nonalkaloid Fraction, and *N*-Oxide Alkaloid Fraction

treatment	change in plasma glucose levels pre- vs post-dose (mg/dL)		body weight, average (g/mouse)		food intake (g/mouse)
	3 h	24 h	0 h	24 h	average
vehicle	−32.9	−7.4	44.5 ± 0.7	44.3 ± 0.7	5.2
metformin[a]	−127.6[b]	18.6	45.5 ± 2.0	45.4 ± 2.2	4.1
aqueous extract[c]	−89.6[b]	−33.9	43.7 ± 0.9	43.7 ± 0.9	5.4
nonalkaloid[c]	−105.1[d]	−13.4	45.2 ± 1.4	45.0 ± 1.4	4.6
N-oxide[c]	−113.8[b]	−113.6[b]	44.9 ± 1.0	44.6 ± 1.0	3.7

[a] Single dose, 250 mg/kg. [b] $p < 0.005$ (paired *t*-test, pre- vs post-dose). [c] Single dose, 1000 mg/kg. [d] $p < 0.05$ (paired *t*-test, pre- vs post-dose).

74.5) indicated one of the hydroxyl groups was attached at this carbon. Finally, long-range correlations between H_2-8 and C-8a and C-9 provided the substructure shown in Figure 1. The position of the remaining hydroxyl group and ring connections in the substructure were guided by the necessity of a tricyclic structure. This requirement was only satisfied with the hydroxyl placed at C-9 (δ 135.5), C-4a joined to C-8a, and C-9 attached to C-9a, generating the gross structure **1a**. Analysis of the EIMS of **1a**, which displayed characteristic peaks at *m*/*z* 234 ($M^+ - CO$), 219 (*m*/*z* 234 − Me), and 201 (*m*/*z* 219 − H_2O), provided support for a hydroxymethyl lactone.



epi-3-Hydroxycacalolide (**1b**) was obtained as an amorphous white solid. The molecular formula, $C_{15}H_{18}O_4$, was determined by HREIMS *m*/*z* 262.1207 (M^+, Δ 0.2 mmu of calc) and ^{13}C and DEPT NMR. The NMR (Tables 2 and 3), IR, and MS data of **1b** were very similar to those of **1a**, and analysis of these data indicate that **1b** is the C-3 epimer of **1a**. Compounds **1a** and **1b** are the 3-hydroxy epimers of cacalolide. (3*S*)-Cacalolide has been isolated previously from *Cacalia delphiniifolia*,[18] and a 1:1 mixture of the C-3 epimers of cacalolide has been reported from *C. adenostyloides*.[17] Further discussion of the stereochemistry of **1a** and **1b** is described below.

Two additional compounds, cacalone (**2a**) and epicacalone (**2b**), were also isolated from the aqueous nonalkaloid fraction. Subsequent large-scale isolation of these compounds utilizing dichloromethane as the extraction solvent also provided the known furanoeremophilane, cacalol (**3**), which was not detected in the aqueous extract. Compounds **2a** and **3** have been isolated previously from *C. decomposita*.[19] The unambiguous structure for **3** was established by several syntheses,[20] and the absolute stereochemistry was reported to be 5*S*.[21] The structure of **2a** was established by the conversion of **3** to **2a**,[22,23] and the structure and 5*S* stereochemistry were verified by X-ray crystallography.[24] A 1:1 mixture of **2a** and **2b** was reported from *C. delphiniifolia*[25] and *C. adenostyloides*.[17] During our current analysis of 1D and 2D NMR data to provide the complete ^{13}C and ^{1}H NMR assignments for **2a**, **2b**, and **3** (see Tables 2 and 3), several revisions in ^{13}C NMR assignments were made for **2a**[23,26] and **3**.[17,27]

Table 2. ^{13}C NMR Data for **1a**, **1b**, **2a**, **2b**, and **3** in $CDCl_3$ (100 MHz)

no.	1a	1b	2a	2b	3
2	178.0	178.7	144.3[a]	144.3	140.7
3	74.5	74.4	120.3	120.2	117.0
3a	124.5	124.7	140.4[a]	140.4	126.0[b,c]
4	124.8	124.9	70.5	72.2	120.1[b,c]
4a	138.5	138.2	161.6[a]	161.6	135.5[b,c]
5	28.6	28.7	27.2	28.5	28.9
6	29.4	29.6	30.1[a,d]	30.2	30.0
7	16.1	16.2	15.6	16.0	16.6
8	23.0	23.2	20.7[a,d]	21.6	22.9
8a	126.6	126.6	130.6	130.6	118.7
9	135.5	134.9	175.1	175.0	136.2[b,c]
9a	136.8	137.0	145.2[a]	145.1	142.1[b,c]
10	24.6	24.0	9.0	8.8	11.2
11	12.6	12.6	25.7	27.2	13.7
12	20.8	20.7	20.7	21.3	21.3

[a] Original assignments reported by Yuste et al.[23]: C-2 (140.5), C-3a (145.3), C-4a (144.3), C-6 (21.6), C-8 (30.3), C-9a (161.6). [b,c] Original assignments reported by Kuroyanagi et al.[17] and Jia and Chen[27]: C-3a (120.1), C-4 (135.5), C-4a (126.1), C-9 (142.4), C-9a (136.4). [d] Original assignments reported for upfield carbons (5−35 ppm) in the acetate derivative of **2a** Jankowski et al.[26]: C-6 (21.5), C-8 (30.5).

The relative stereochemistry and solution conformations of **1a** and **1b** were assigned using a combination of proton coupling constants of diastereotopic protons H_2-8, a key ROESY correlation between H_3-10 and H_3-12, and molecular mechanics calculations (PCMODEL). Two isoenergetic half-chair conformations were calculated for both **1a** and **1b** due to ring reversal in the cyclohexene ring, placing H_3-12 in a pseudoaxial position in one conformer (**1a-ax**, **1b-ax**) and pseudoequatorial (**1a-eq**, **1b-eq**) in the other (Figure 2, Table 4). Superimposition of **1a-ax** with **1a-eq**, as well as **1b-ax** with **1b-eq**, revealed that most heavy atoms occupied the same space, except for C-6 and C-7 due to torsion about the C-5−C-6−C-7−C-8 dihedral angle. The equivalent calculated energies for each pair of conformers

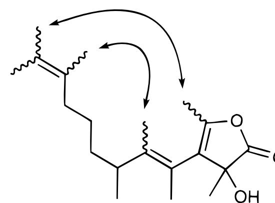


Figure 1. Substructure for **1a**.

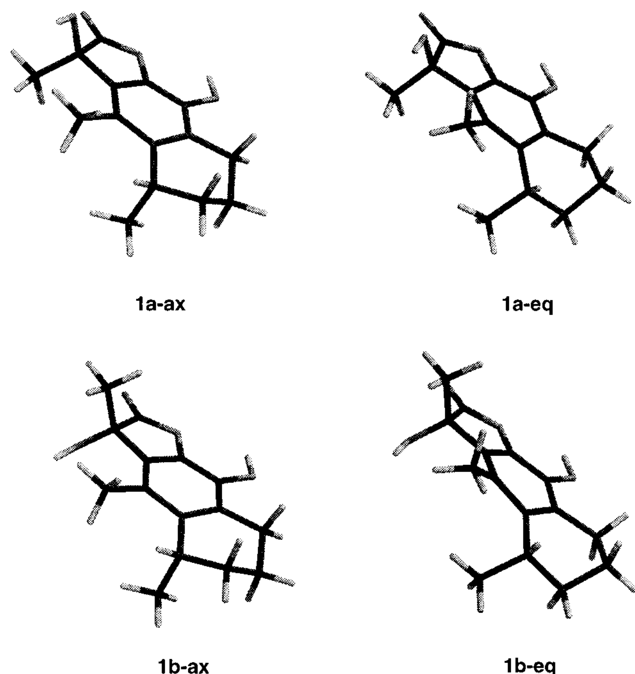


Figure 2. Energy minimized conformations for **1a** and **1b**.

suggested they may exist in equilibrium in solution; however, examination of the ^{1}H NMR of **1a** and **1b** in $CDCl_3$ and C_6D_6 indicated that one dominant conformation exists in solution for each compound. The diastereotopic shift difference between Hα-8 and Hβ-8 in **1a** ($\Delta\delta = 0.46$ ppm), along with the divergent vicinal coupling values 11.7, 7.4 Hz for Hα-8 and 5.4, 1.1 Hz for Hβ-8 in C_6D_6, indicated that the cyclohexene ring was rigid.[28] Likewise, the diastereotopic shift difference between Hα-8 and Hβ-8 in **1b** ($\Delta\delta = 0.50$ ppm) and the divergent vicinal coupling values 11.0, 7.6 Hz for Hα-8 and 5.0, <1 Hz for Hβ-8 in C_6D_6 suggested that the cyclohexene ring was also rigid in this diastereomer. The **1a** and **1b** solution conformations were assigned as **1a-ax** and **1b-ax** based on the better fit of the observed coupling constants for H-8 protons with those calculated for **1a-ax** and **1b-ax** (Table 5). The relative stereochemistry in **1a** was assigned as 3*R**,5*S** (and 3*S**,5*S** in **1b**), based on a key ROESY correlation observed between H_3-10 and H_3-12 in **1a** and not **1b**, suggesting that these methyls in **1a** are on the same face of the molecule and in closer proximity to each other versus their opposing arrangement in **1b**.

Examination of the H_2-8 diastereotopic shift differences and the associated coupling constants for compounds **1a**, **1b**, **2a**, and **2b** in Table 3 suggested that they all exist in a similar conformation in solution. Conformational analysis of **2a** and **2b** using a similar strategy as outlined above indicated both diastereomers exist in solution in the H_3-12 pseudoaxial (**2a-ax** and **2b-ax**) conformation. A comparison between the observed proton coupling constants for **2a** and **2b** with those calculated for **2a-ax**/**2a-eq** and **2b-ax**/**2b-eq**, respectively, indicated the best fit with **2a-ax** and **2b-ax**, particularly because the calculated H-6 axial couplings fit the observed data with H-5 in a pseudoequatorial position in **2a-ax** and **2b-ax**, whereas H-5 is pseudoaxial in **2a-eq** and **2b-eq** and would require an additional 12 Hz coupling, which was not observed (Table 6). Thus, **2a** exists in the **2a-ax** conformation in solution as well as in the solid state.[24]

When a 1:1 mixture of **1a**/**1b** and **2a**, **2b**, and **3** were dosed at 1.09 mmol/kg (single dose) in *ob*/*ob* mice, **1a**/**1b** and **3** lowered plasma glucose levels along with the positive control group receiving metformin (1.5 mmol/kg), a drug used clinically to treat type 2 diabetes (Table 7). Compounds **2a** and **2b** were inactive. Compounds **1a** and **1b** were not tested separately because of the difficulty of isolating sufficient material required for in vivo testing. The mechanism of action of **1a**, **1b**, and **3** in lowering blood glucose is unknown. However, the fact that **3** inhibits electron transport and oxidative phosphorylation[29] may play a role in the antihyperglycemic effect of **3**. Recent studies have shown that inhibitors of oxidative phosphorylation stimulate glucose transport via induction of GLUT1 mRNA and GLUT1 protein in Clone 9 cells.[30] GLUTs are a set of homologous glycoproteins that are involved in the passive transmembrane transport of glucose.[31] Stimulation of glucose transport by inhibitors of oxidative phosphorylation leads to enhanced glycolysis and recovery of normal levels of cellular ATP.

P. decompositum is taken orally in Mexico and the Southwestern United States for the treatment of diabetes, among other ailments, and both aqueous and ethanolic preparations are used.[14] We prepared both extracts and found the presence of **1a**, **1b**, and **3** in the ethanolic extract, and **1a** and **1b** in the aqueous extract, along with the pyrrolizidine alkaloids in both extracts. Our results suggest that **1a**, **1b**, and **3** contribute, at least in part, to the treatment of diabetes. In addition, the antihyperglycemic effect of the *N*-oxide pyrrolizidine alkaloid fraction suggests that the pyrrolizidines present may also be responsible for the glucose lowering ability of this herbal treatment for diabetes. However, the preliminary work done on the detection of 1,2-unsaturated pyrrolizidines in *P. decompositum*, and their potential for hepatotoxicity, suggests that this plant should not be used as an herbal treatment until safety studies are completed. Investigations in our laboratory of the relationship between hepatotoxicity and antihyperglycemia of pyrrolizidine alkaloids with and without 1,2-unsaturation are underway. A paper on the total synthesis of racemic cacalol has been submitted for publication.[32]

Experimental Section

General Experimental Procedures. Analytical HPLC was performed on a Hitachi D-6500 equipped with a L-6200A pump, AS-2000 autosampler, L-4500 A diode array detector, and Sedex 55 light-scattering detector connected in parallel. Semipreparative HPLC was performed on a Hitachi D-6500 Chromatography Data Station equipped with a Waters 600 pump controller and L-4500 A diode array detector. Preparative HPLC was carried out with a Rainin Dynamax HPLC system equipped with a Dynamax diode array detector (model PDA-1) and solvent delivery pumps (model SD-1). NMR spectra were recorded on a Varian Unity Plus 400 or a Varian Unity 400 spectrometer. 2D experiments were acquired and processed using routine parameters. MS were recorded on a Kratos MS-50 in high-resolution power electron impact scanning mode, 70 eV. Other instrumentation included a Perkin−Elmer 1600 series FTIR, Perkin−Elmer UV−vis spectrometer, and a JASCO DIP370 polarimeter. Molecular modeling was carried out with the PCMODEL program, version 3.0 (Serena Software). Molecular mechanics calculations used the MMX forcefield with charge−charge electrostatics.

Plant Material. Roots of *P. decompositum* were purchased and collected in Zaragosa district, Muero Leon, Mexico, and identified by A. Pool, Missouri Botanical Gardens. Voucher specimens (no. 12 and 9−5a) were deposited in the reference collection at the Department of Ethnobotany and Conservation, Shaman Pharmaceuticals, Inc.

Biological Assay. The genetically altered obese diabetic C57BL-6J *ob*/*ob* mice used in the bioassay as a model for type 2 diabetes are described in detail elsewhere.[33]

Table 3. ^{1}H NMR Data for **1a**, **1b**, **2a**, **2b**, and **3** in $CDCl_3$ (400 MHz)

no.	**1a**	**1b**	**2a**	**2b**	**3**
2			7.31 m	7.34 q (1.2)	7.27 s
5	3.01 m	2.95 m	3.12 m	2.87 m	3.28 m
6α	1.7 m	1.52 m	1.47 tt (13, 4)	1.58 tt (12, 4)	1.84 m
6β	1.7 m	1.7 m	1.68 m	1.70 dq (12, 3)	1.84 m
7	1.77 m	1.7 m	1.72−1.83 m	1.75−1.8 m	1.9 m
8α	2.45 ddd (18, 10, 8)	2.24 m	2.37 ddd (19, 8, 8)	2.33 ddd (18, 9, 9)	2.68 ddd (18, 11.2, 6.8)
8β	2.83 br dd (18, 6)	2.75 br d (18)	2.45 ddd (19, 8.4, 2.8)	2.54 ddd (18, 6.8, 2.4)	3.04 ddd (17.2, 5.2, 1.2)
10	1.69 s	1.67 s	2.23 d (1.2)	2.23 d (1.2)	2.42 s
11	2.29 s	2.28 s	1.68 s	1.67 s	2.56 s
12	1.10 d (6.8)	1.05 d (7.2)	1.26 d (6.8)	1.31 d (7.2)	1.24 d (7.2)

Table 4. Minimized Energies (kcal/mol) for **1a**, **1b**, **2a**, **2b** Conformers

	1a-ax	**1a-eq**	**1b-ax**	**1b-eq**	**2a-ax**	**2a-eq**	**2b-ax**	**2b-eq**
E min	26.3	25.7	25.1	25.1	26.7	26.1	25.0	26.2

Table 5. Observed (in C_6D_6) and Calculated Proton Coupling Constants (J in Hz) for **1a** and **1b**

	1a obsd	**1a-ax** calcd	**1a-eq** calcd	**1b** obsd	**1b-ax** calcd	**1b-eq** calcd
$J_{7eq-8ax}$	7.4	5.9	3.6	7.6	7.1	3.5
$J_{7ax-8ax}$	11.7	12.2	13.2	11.0	11.3	13.2
$J_{7eq-8eq}$	1.1	1.3	2.8	<1	0.7	2.9
$J_{7ax-8eq}$	5.4	5.6	3.3	5.0	6.9	3.2

Table 6. Observed (in $CDCl_3$) and Calculated Proton Coupling Constants (J in Hz) for **2a** and **2b**

	2a obsd	**2a-ax** calcd	**2a-eq** calcd	**2b** obsd	**2b-ax** calcd	**2b-eq** calcd
$J_{7eq-8ax}$	8	8.2	4.8	9	7.1	5.2
$J_{7ax-8ax}$	8	10.2	12.8	9	11.3	12.7
$J_{7eq-8eq}$	2.8	0.4	1.8	2.4	0.7	1.6
$J_{7ax-8eq}$	8.4	8.2	4.6	6.8	7.0	4.8
$J_{6ax-7ax}$	13	13.2	13.2	12	13.2	13.2
$J_{6ax-7eq}$	4	3.0	2.5	4	2.7	2.4
$J_{6ax-5eq}$	4	3.5		4	4.0	
$J_{6ax-5ax}$			12.0			12.1

Extraction and Isolation. The ground roots of *P. decompositum* (500 g, voucher specimen no. 12) were extracted in 55 °C H_2O for 24 h, filtered, and then dried to yield a H_2O extract (77.4 g). A portion of this extract (15.1 g) was dissolved in H_2O (90 mL), followed by the addition of 600 mL of isopropyl alcohol. The mixture was left to stand at 4 °C for 12 h, the solids were then removed by decantation and the supernatant dried (6.45 g). A portion of the dried supernatant (5.5 g) was dissolved in 1N HCl (55 mL) and extracted with CH_2Cl_2 (3 × 70 mL). The CH_2Cl_2 phase was washed with 0.5N HCl (3 × 50 mL) and then H_2O (2 × 100 mL). Final purification of a 132-mg portion of the CH_2Cl_2 phase (163 mg) by HPLC (PRP-1, Hamilton, 10 μm, 20 × 250 mm, MeCN−H_2O gradient, 16 mL/min followed by ODS-AQ, YMC Inc., 5 μm, 20 × 200 mm, MeCN−H_2O gradient, 12 mL/min) gave 2.3 mg of a 1:1 mixture of **1a** and **1b**, 1 mg of **2a**, and 15 mg of **2b**. The acidic phase was made basic (pH 9) with 2N NH_4OH and extracted with CH_2Cl_2 (3 × 70 mL) to yield 89 mg of a free-base alkaloid fraction that tested positive for pyrrolizidine alkaloids by TLC on Si gel 60 developed in $CHCl_3$−MeOH−NH_4OH (165:31:4) followed by visual detection of three magenta spots using *o*-chloranil and Ehrlich's reagent. The remaining basic phase was then neutralized and eluted on Diaion HP-20 (40 × 70 mm column size) in H_2O followed by MeOH. The presence of *N*-oxide pyrrolizidine alkaloids, similar to those detected in the free-base alkaloid fraction, were detected in the MeOH eluent (798 mg). The MeOH eluent (2 mg) was dissolved in 2 mL of 0.5N HCl, and a catalytic amount of Zn was added. The solution was stirred for 1 h, neutralized with saturated solution of $NaHCO_3$, and then extracted with CH_2Cl_2. This reduced material along with the free-base alkaloid fraction was developed on TLC, followed by visual detection using *o*-chloranil and Ehrlich's reagent; three magenta spots with the same R_f were observed in both samples.

An organic extraction was employed to isolate compounds for in vivo testing. Ground roots of *P. decompositum* (5 kg, voucher specimen no. 9−5a) were extracted with CH_2Cl_2 for 6 h at room temperature. The extract (321.4 g) was dissolved in EtOH−H_2O, 9:1 (2 L) and extracted with petroleum ether (4 × 400 mL). The alcohol layer residue (149.3 g) was adsorbed onto 1.5 L of Si gel (70−230 mesh, 60 Å) and then added onto 1 L of Si gel (15.5 × 12 cm final bed size) and fractionated by vacuum flash chromatography. Three fractions (3.5 L/fraction) were eluted with hexane−EtOAc, 85:15, followed by a fourth with 80:20 of the same solvent system (8 L). The first fraction (38.5 g) was crystallized in petroleum ether, and 14.8 g of **3** was obtained. Purification of the fourth fraction (24.1 g) by sequential HPLC (PRP-1, Hamilton, 50 × 250 mm; Primesphere ODS HC 10 μm, 50 × 250 mm, 50 × 30 mm precolumn, 70:30 MeCN−H_2O, 50 mL/min; Primesphere ODS HC 10 μ, 50 × 250 mm, 50 × 30 mm precolumn, 60:40 MeCN−H_2O, 50 mL/min) yielded 1.41 g of a 1:1 mixture of **1a/1b**, 0.41 g of **2a**, and 1.43 g of **2b**. Small amounts of **1a** and **1b** were isolated from the 1:1 mixture by HPLC (phenyl, YMC Inc., 5 μm, 20 × 250 mm, MeOH−H_2O gradient).

3-Hydroxycacalolide (1a): amorphous white solid; $[\alpha]_D$ −18° (c 0.59, CH_2Cl_2); UV (EtOH) λ_{max} (log ϵ) 295 (3.37); IR (film) cm^{-1} 3374, 2930, 1798, 1635, 1443, 1336, 754; ^{1}H and

Table 7. Change in Glucose Level, Body Weight, and Food Consumption in *ob*/*ob* Mice Receiving Vehicle, Positive Control, **1a**/**1b**, **2a**, **2b**, and **3**

treatment	change in plasma glucose levels pre- vs post-dose (mg/dL)		body weight, average (g/mouse)		food intake (g/mouse)
	3 h	24 h	0 h	24 h	average
vehicle	−35.4	120.4	44.1 ± 0.8	44.1 ± 0.8	5.1
metformin[a]	−151.8[d]	76.1	43.5 ± 0.4	43.2 ± 0.5	4.5
1a/1b[b]	−110.1[d]	−35.5	42.9 ± 1.2	42.6 ± 1.2	4.8
2a[c]	−3.0	29.5	41.6 ± 0.9	41.4 ± 0.9	4.8
2b[c]	−42.4	30.9	44.1 ± 1.6	43.3 ± 1.5	4.0
3[c]	−124.0[d]	48.3	44.1 ± 0.8	43.5 ± 0.7	4.1

[a] Single dose, 1.5 mmol/kg. [b] Single dose of a 1:1 mixture, 1.09 mmol/kg. [c] Single dose, 1.09 mmol/kg. [d] $p < 0.005$ (ANOVA).

^{13}C NMR, see Tables 2 and 3; EIMS *m/z* 262 M$^+$ (19), 234 (43), 219 (100), 201 (7); HREIMS *m/z* 262.1216 (calcd for $C_{15}H_{18}O_4$ 262.1205).

***epi*-3-Hydroxycacalolide (1b):** amorphous white solid; $[\alpha]_D$ +35° (*c* 1.00, CH_2Cl_2); UV (EtOH) λ_{max} (log ϵ) 295 (3.37); IR (film) cm^{-1} 3380, 2931, 2866, 1799, 1634, 1425, 1336, 755; ^{1}H and ^{13}C NMR, see Tables 2 and 3; EIMS *m/z* 262 M$^+$ (18), 234 (43), 219 (100), 201 (19); HREIMS *m/z* 262.1207 (calcd for $C_{15}H_{18}O_4$ 262.1205).

Cacalone (2a): white solid; mp 139−141 °C (lit. mp 139−141 °C);[25] $[\alpha]_D$ +95° (*c* 1.00, CH_2Cl_2), lit. $[\alpha]_D$ +87° (*c* 1.00, $CHCl_3$);[25] IR (film) cm^{-1} 3390, 2936, 1652, 1614, 1538, 1419; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HREIMS *m/z* 246.1252 (calcd for $C_{15}H_{18}O_3$ 246.1256).

Epicacalone (2b): white solid; mp 128−130 °C (lit. mp 129.5−131 °C);[25] $[\alpha]_D$ +81° (*c* 1.00, CH_2Cl_2), lit. $[\alpha]_D$ +95° (*c* 0.980, $CHCl_3$);[25] IR (film) cm^{-1} 3413, 2934, 1652, 1614, 1536, 1420; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HREIMS *m/z* 246.1254 (calcd for $C_{15}H_{18}O_3$ 246.1256).

Cacalol (3): white solid; IR (film) cm^{-1} 3507, 2928, 2867, 1629, 1450, 1406, 1112; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HREIMS *m/z* 230.1309 (calcd for $C_{15}H_{18}O_2$ 230.1307).

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