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ARRIVACINS, NOVEL PSEUDOGUAIANOLIDE ESTERS WITH POTENT ANGIOTENSIN II BINDING ACTIVITY FROM *AMBROSIA PSILOSTACHYA*

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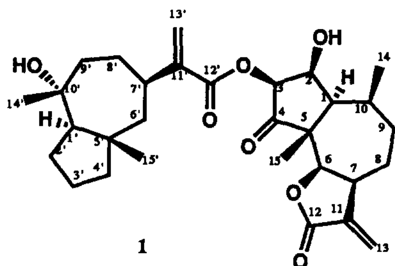
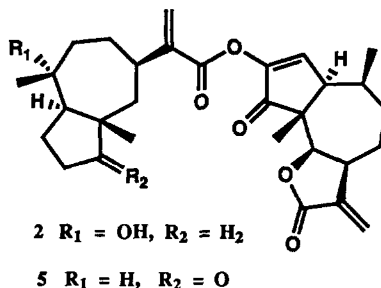
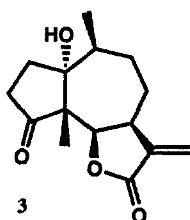
Abstract. Two novel pseudoguaianolide esters, arrivacins A (**1**) and B (**2**), have been isolated from the methylene chloride extract of *Ambrosia psilostachya* collected in Arrivaca Cienega, Arizona. The structures of the arrivacins, which are examples of sesquiterpene dimers formed from the condensation of two different pseudoguaianolide sesquiterpenes, were determined by analysis of the spectral data and chemical interconversion. Arrivacins A and B show potent binding to angiotensin II receptors in bovine adrenal membranes.

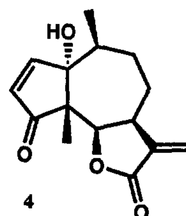
Angiotensin II (AII) is an octapeptide that is generated by the proteolytic processing of angiotensin I by angiotensin converting enzyme (ACE) and plays a central role in control of blood pressure.[1] Dysfunction of the renin-angiotensin system is a key factor in cardiovascular and renal disease. Inhibitors of ACE represent a prevalent class of therapeutics for hypertension;[2] however, selective antagonism of the A II receptor may allow more precise therapeutic intervention. While potent receptor antagonists for A II have been found and shown to have a clear pharmacological utility (e.g. saralasin), none have yet been developed with sufficient therapeutic benefit. In the course of our search for natural products that are selective A II receptor antagonists, it was observed that the CH_2Cl_2 extract of *Ambrosia psilostachya* showed potent inhibition (IC_{50} = 28 µg/mL) of the binding of ^{125}I labeled angiotensin II to receptors prepared from bovine adrenal membranes.

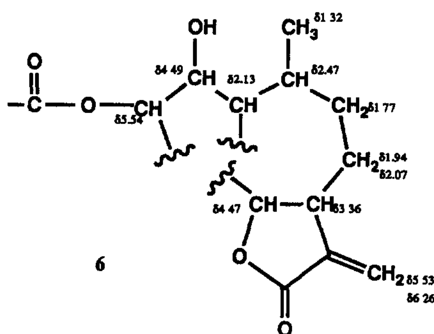
Ambrosia psilostachya DC (Asteraceae, Heliantheae, Ambrosiinae) is a perennial weed belonging to the genus of ragweeds. *A. psilostachya* is a widespread species growing along streams, on plains, in fields, on hillsides and roadsides from Washington to Saskatchewan, in Illinois and south to California, Arizona, Texas and northern Mexico. In Arizona, where the sample used in this study was collected, it is abundant in the Apache, Navajo, Coconino, Cochise and Pima counties where it grows at 2,500 to 7,000 feet elevations. The genus *Ambrosia* is known to produce numerous sesquiterpene lactones of the germacranolide, psilostachyanolide, pseudoguaianolide, ambrosanolide and eudesmanolide types.[3] *A. psilostachya* has been shown to produce the pseudoguaianolides coronopilin (**3**),[4] parthenin (**4**),[5-6] ambrosin,[6] damsin,[6] 3-hydroxy damsin,[7] cumanin,[5,8] cumanin 3-acetate,[5] cumanin diacetate,[5] ambrosiol,[9]and desacetyl-confertiflorin[3] (which bear the ambrosanolide stereochemistry); the cleaved pseudoguaianolides psilostachyins,[10-12] and the germacranolide isabelin.[13] To our knowledge the only previous example of a naturally occuring ester formed from the condensation of two pseudoguaianolide sesquiterpenes was the discovery of the ester of damsinic acid and 3-hydroxyambrosin (**5**) in *Ambrosia hispida.*[14]

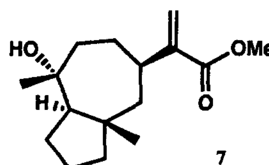












Using an AII *in vitro* binding assay to guide isolation, the CH_2Cl_2 extract of *A. psilostachya* was fractionated using a series of normal and reverse phase column and HPLC chromatographic procedures to yield arrivacin A (**1**, 0.01 % dry wt.) and arrivacin B (**2**, 0.002% dry wt.) as the AII binding inhibitory components of the crude extract. In addition to these two AII active metabolites, the two known pseudoguaianolide lactones coronopilin (**3**, 0.15% dry wt.) and parthenin (**4**, 0.03% dry wt.) were isolated and their structures determined by comparison with published spectral data.[12-13] Neither **3** nor **4** inhibited receptor binding of AII.

The NMR data for **1** and **2** (Tables 1 & 2) were very similar and the molecular formulae determined by high resolution mass spectrometry indicated the two arrivacins differed in elemental composition by the elements of water. The molecular formula of **1** was established by high resolution EIMS as $C_{30}H_{42}O_7$ which implies the presence of 10 double bond equivalents. Although the formula of **1** was suggestive of a highly oxygenated triterpene, closer analysis of the NMR and MS data suggested that **1** was a sesquiterpene dimer. The low field ^1H

NMR region exhibited two doublets typical of an α-methylene-γ-lactone moiety common to the sesquiterpene lactones well-known from the genus *Ambrosia*. The presence of this unsaturated lactone moiety was confirmed by comparison of other spectral data with the two known pseudoguaianolides 3 and 4 (Table 3). Further support for a sesquiterpene dimer was provided by exact mass measurements on the intense fragment ions which also suggested the presence of an ester link between the two C_{15} halves (Figure 1). Other observed fragments correspond to through-ring cleavages and do not substantially aid structural elucidation. Further analysis of the downfield NMR region suggested the evidence of another α-methylene unsaturated ester (CMR δ 123.7t, 145.0s, 166.3s and PMR δ 6.23bs, 5.57bs) and a ketone functionality (CMR δ 209.6s). The above data accounted for all sp^2 carbons in the CMR and for 6 double bond equivalents, therefore arrivacin A (**1**) must have 4 rings in addition to the α-methylene-γ-lactone. The α-methylene group of this γ-lactone provided a convenient starting point for analysis of the ^1H-^1H COSY NMR spectrum (Table 4).

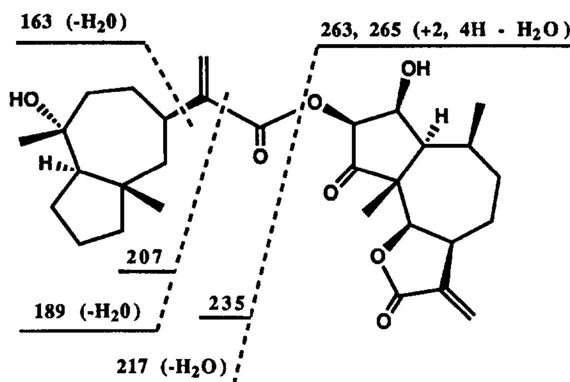


Figure 1. Mass spectral (DCI, isobutane, positive ion mode) fragmentation of arrivacin A (**1**).

The ^1H-^{13}C correlation spectrum of **1** showed an exocyclic methylene carbon (t, δ120.7) attached to two protons, at δ5.53 and δ6.26, which were observed in the COSY spectrum to be coupled to a methine at δ3.36. This methine, in turn, was observed to be coupled to a methine proton on a carbon bearing oxygen (δ4.47 and δ81.4 ppm) which was one terminus of a network of coupled protons and to two upfield proton signals at δ1.94 and δ2.07. The geminal nature of these two upfield protons was confirmed by their mutual correlation to a ^{13}C triplet at δ25.7. The δ1.94 proton was further coupled to an upfield methylene pair at δ1.77 which in turn was coupled to a methine proton at δ2.47 ppm. This methine proton at δ2.47 was likewise coupled to the C-14 methyl doublet (δ1.32) and to the C-1 bridgehead proton at δ2.13 ppm. The C-1 bridgehead proton was also coupled to a downfield methine (δ4.49) on carbon bearing oxygen (δ73.7) which was further coupled to another methine proton (δ5.54) on carbon bearing oxygen (δ78.2). From the relative chemical shift of these two downfield protons, the δ4.49 proton at C-2 indicates a hydroxyl functionality at C-2 while the δ5.54 proton at C-3 indicates an ester attachment at this carbon. This completed the first spin system and gave the partial structure **6**, which permitted determination of the alcohol portion of the sesquiterpene dimer **1**.

Table 1. Proton NMR data [δH (multiplicity,intensity, *J*-Hz)]* for arrivacins A & B and esters **5** & **7**

Atom	Arrivacin A (1)	Arrivacin B (2)	(5)§	Methyl Ester (7)
H-1	2.13 (dd, 1H, 4.0, 3.0)	2.97 (dd, 1H, 5.6, 2.1)	3.00 (dd, 1H, 6, 2)	-
H-2	4.49 (dd, 1H, 4.5, 3.0)	7.15 (d, 1H, 2.1)	7.17 (d, 1H, 2)	-
H-3	5.54 (d, 1H, 4.5)	-	-	-
H-6	4.47 (d, 1H, 8.5)	4.66 (d, 1H, 8.5)	4.67 (d, 1H, 9)	-
H-7	3.36 (m, 1H)	3.45 (m, 1H)	3.46 (m, 1H)	-
H-8a	2.07 (dd, 1H, 16.0,2.0)	2.24 (m, 1H)	2.25 (m, 1H)	-
H-8b	1.94 (m, 1H)	1.93 (m, 1H)	1.92 (m, 1H)	-
H-9a	1.77 (m, 2H)	1.89 (m, 1H)	1.80 (m, 1H)	-
H-9b	1.77 (m, 2H)	1.75 (m, 1H)	1.70 (m, 1H)	-
H-10	2.47 (m, 1H)	2.40 (bd, 1H, 7.5)	2.42 (m, 2H)	-
H-13a	6.26 (d, 1H, 3.5)	6.30 (d, 1H, 3.5)	6.30 (d, 1H, 3.5)	-
H-13b	5.53 (d, 1H, 3.0)	5.55 (d, 1H, 3.1)	5.51 (d, 1H, 3.2)	-
H-14	1.32 (d, 3H, 7.0)	1.08 (d, 3H, 7.5)	1.09 (d, 3H, 7)	-
H-15	1.36 (s, 3H)	1.28 (s, 3H)	1.28 (s, 3H)	-
H-1'	1.26 (m, 1H)	1.32 (m, 1H)	signal obscured	1.28 (m, 1H)
H-2a'	1.78 (m, 1H)	1.80 (m, 1H)	signal obscured	1.79 (m, 1H)
H-2b'	1.37 (m, 1H)	1.36 (m, 1H)	signal obscured	1.34 (m, 1H)
H-3'	1.54 (m, 2H)	1.55 (m, 2H)	2.4 (ddd,1H) & 2.2 (m,1H)	1.54 (m, 2H)
H-4a'	1.41 (m, 1H)	1.40 (m, 1H)	-	1.39 (m, 1H)
H-4b'	1.13 (m, 1H)	1.09 (m, 1H)	-	1.17 (m, 1H)
H-6a'	2.00 (m, 1H)	1.98 (m, 1H)	signal obscured	1.90 (bd, 1H, 12.0)
H-6b'	1.08 (m, 1H)	1.22 (m, 1H)	signal obscured	1.20 (bd, 1H, 12.0)
H-7'	2.50 (m, 1H)	2.53 (m, 1H)	2.77 (m, 1H)	2.52 (btt, 1H, 11.5,3.5)
H-8a'	1.62 (m, 1H)	1.66 (m, 1H)	signal obscured	1.59 (m, 1H)
H-8b'	1.49 (m, 1H)	1.40 (m, 1H)	signal obscured	1.43 (m, 1H)
H-9a'	1.50 (m, 1H)	1.47 (m, 1H)	signal obscured	1.65 (m, 1H)
H-9b'	1.30 (m, 1H)	1.27 (m, 1H)	signal obscured	1.28 (m, 1H)
H-13a'	6.23 (bs, 1H)	6.35 (bs, 1H)	6.33 (bs, 1H)	6.13 (bs, 1H)
H-13b'	5.57 (bs, 1H)	5.74 (bs, 1H)	5.73 (bs, 1H)	5.56 (bs, 1H)
H-14'	1.11 (s, 3H)	1.11 (s, 3H)	1.07 (d, 3H, 7)	1.10 (s, 3H)
H-15'	0.90 (s, 3H)	0.91 (s, 3H)	1.07 (s, 3H)	0.91 (s, 3H)
-OMe	-	-	-	3.76 (s, 3H)

*Chemical shifts determined from a detailed analysis of ^1H NMR and ^{13}C-^1H heteronuclear correlation spectra.
§Taken from W. Herz *et al.*[14]

The acid portion of **1** was likewise determined by starting with the C-13' exocyclic methylene carbon and attached protons. Methanolysis of arrivacin A (**1**) gave the methyl ester **7** of the acid portion of **1**, but the presumably unstable diol corresponding to the alcohol portion of **1** could not be isolated from the reaction mixture. The NMR data for the methyl ester **7** (Tables 1 & 2) were consistent with the proposed structure further confirming the structure of arrivacin A (**1**) and providing a model compound for NOE studies to help determine stereochemistry.

Biogenetic considerations suggested that arrivacin A (**1**) should possess an ambrosanolide skeleton common to sesquiterpene lactones isolated from the subtribe Ambrosiinae, Asteraceae.[15] Pseudoguaianolide sesquiterpenes with this skeleton possess a trans-fused 5,7-ring system with C-10 and C-5 β methyl groups. The lactone ring is cis-fused with H-6 and H-7 in the α orientation. Likewise C-14', C-15' and the unsaturated ester group at C-7' should all be in the β orientation and the C-1', C-5' ring junction should be trans. These and other stereochemical assignments were confirmed by a series of difference NOE experiments on both arrivacin A (**1**) and the methyl ester **7** derived from **1**. NOE difference experiments on arrivacin A (**1**) showed selective enhancement of H-3, H-1, H-7 and H-10 when the overlapping signals of H-2 and H-6 were irradiated, indicating that all of these protons had the α orientation. Further evidence supporting the β orientation of the C-2 hydroxyl group came from a comparison of the ^1H NMR data for arrivacin A (**1**) and the published NMR data for

ivoxanthin (8) and bipinnatin (9).[16] The deshielding effect produced on C-14 and C-15 by the syn-axial interaction of the β-OH at C-2 in bipinnatin (9) is also observed in arrivacin A (1).

Table 2. Carbon NMR data for arrivacins A and B and esters 5 and 7

Atom	Arrivacin A (1)	Arrivacin B (2)	(5)§	Methyl Ester (6)
C-1	45.3 d	43.0 d	42.8 d	-
C-2	73.7 d	143.3 d	143.8 d	-
C-3	78.2 d	144.4 s	145.9 s	-
C-4	209.6 s	202.2 s	201.8 s	-
C-5	52.6 s	55.3 s	55.2 s	-
C-6	81.4 d	79.9 d	79.9 s	-
C-7	43.9 d	44.5 d	44.4 d	-
C-8	25.7 t	24.8 t	24.7 t	-
C-9	34.7 t	29.9 t	29.9 t	-
C-10	35.0 d	34.0 d	33.8 d	-
C-11	139.4 s	138.2 s	138.5 s	-
C-12	169.6 s	170.2 s	170.1 s	-
C-13	120.7 t	119.9 t	119.4 t	-
C-14	17.4 q	17.2 q	17.2 q	-
C-15	17.2 q	17.2 q	17.2 q	-
C-1'	54.8 d	55.2 d	46.2 d	55.2 d
C-2'	43.4 t	43.4 t	22.5 t	43.6 t
C-3'	20.2 t	20.1 t	35.8 t	20.2 t
C-4'	41.0 t	41.0 t	220.5 s	41.1 t
C-5'	34.7 s	34.6 s	50.9 s	34.7 s
C-6'	27.3 t	26.5 t	38.1 t	27.5 t
C-7'	40.8 d	40.7 d	37.0 d	40.7 d
C-8'	26.5 t	27.2 t	34.0 t	26.6 t
C-9'	44.4 t	44.5 t	30.8 t	44.6 t
C-10'	72.5 s	72.0 s	35.7 d	72.1 s
C-11'	145.0 s	147.0 s	146.8 s	146.0 s
C-12'	166.3 s	164.1 s	163.6 s	175.9 s
C-13'	123.7 t	125.3 t	124.7 t	122.4 t
C-14'	22.9 q	22.6 q	21.0 q	22.7 q
C-15'	18.7 q	18.7 q	17.2 q	18.8 q
-OMe	-	-	-	51.7 q

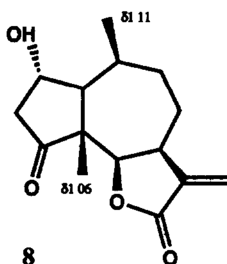
§Taken from W. Herz *et al.*[14]

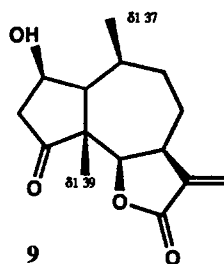
Table 3. Spectral data indicating the presence of a α-methylene-γ-lactone moiety in 1 - 4.

Spectra	Arrivacin A (1)	Coronopilin (3)	Arrivacin B (2)	Parthenin (4)
IR (KBr)	1657 cm^{-1}	1657 cm^{-1}	1650 cm^{-1}	1658 cm^{-1}
	1759 cm-1	1740 cm-1	1748 cm-1	1747 cm-1
PMR				
H_{13a}	6.26 d (3.5Hz)	6.20 d (2.8Hz)	6.30 d (3.5Hz)	6.27 d (2.5Hz)
H_{13b}	5.53 d (3.0Hz)	5.57 d (2.4Hz)	5.55 d (3.1 Hz)	5.59 d (2.5Hz)
H_6	4.47 d (8.5Hz)	4.89 d (8.1Hz)	4.66 d (8.5Hz)	5.00 d (7.8Hz)
H_7	3.36 m	3.34 m	3.45 m	3.49 m
CMR				
C_{11}	139.4 s	141.0 s	144.4 s	140.4 s
C_{12}	169.6 s	170.6 s	170.2 s	170.6 s
C_{13}	120.7 t	121.4 t	119.9 t	121.6 s
C_6	81.4 d	79.6 d	79.9 d	78.6 s
C_7	43.9 d	44.6 d	44.5 d	44.3 d
UV(λmax)	212 nm	215 nm	214 nm	214 nm

Table 4. Proton - proton correlations of arrivacin A (1) from homonuclear COSY spectra.

Proton	Indicated Couplings	Proton	Indicated Couplings
H-1	H-2, H-10	H-2b'	H-1', H-2a', H_2-3'
H-2	H-1, H-3	H_2-3'	H-2a', H-2b', H-4a', H-4b'
H-3	H-2	H-4a'	H_2-3', H-4b'
H-6	H-7	H-4b'	H_2-3', H-4a'
H-7	H-6, H-8a, H-8b, H-13a, H-13b	H-6a'	H-6b', H-7'
H-8a	H-7, H-8b	H-6b'	H-6a', H-7'
H-8b	H-7, H-8a, H_2-9	H-7'	H-6a',H-6b',H-8a',H-8b',H-13b'
H_2-9	H-8a, H-10	H-8a'	H-7', H-8b', H-9b'
H-10	H-1, H-9, H-14	H-8b'	H-7', H-8a', H-9b'
H-13a	H-7	H-9a'	No Correlations Observed
H-13b	H-7	H-9b'	H-8a', H-8b'
H_3-14	H-10	H-13a'	H-13b'
H_3-15	No Correlations Observed	H-13b'	H-7'(weak), H-13a'
H-1'	H-2b', H-15' (weak)	H_3-14'	No Correlations Observed
H-2a'	H-2b', H_2-3'	H_3-15'	H-1' (weak)





The stereochemistry of the acid portion of arrivacin A (1) was confirmed by NOE experiments on the methyl ester 7. NOE difference experiments on the methyl ester 7 showed selective enhancement of the C-14' and C-15' methyl groups when the exocyclic olefin proton at δ6.13 was irradiated. Dreiding models indicated this was only possible when C-14', C-15' and the C-7',C-11' bond were all in the β orientation.

High resolution FABMS established the molecular formula of arrivacin B (2) as $C_{30}H_{40}O_6$ which differs from arrivacin A (1) by the loss of H_2O. The NMR spectrum of arrivacin B (2) was very similar to that of arrivacin A (1). The major difference was the absence in 2 of the two oxygen bearing methines at δ73.7 and δ78.2 in the CMR and the corresponding proton signals at δ4.49 and δ5.54 in the PMR. These were replaced by two additional olefinic carbons at δ143.3(d) and δ138.2(s), and an additional olefinic proton at δ7.15 (d, J = 2.1 Hz). These data suggested that arrivacin B was the dehydrated form of arrivacin A shown in structure 2. The structure of 2 was confirmed by dehydration of arrivacin A (1) to give 2 identical in all respects to the natural product.

Figure 2 shows the binding curve for arrivacin A (1) and demonstrates that 1 clearly inhibits angiotensin II binding to receptors from bovine adrenal cortex. Analysis of the data indicated an IC_{50} = 3 μM. Similarly, arrivacin B (2) was found to be a weaker inhibitor with IC_{50} = 13 μM (data not shown).

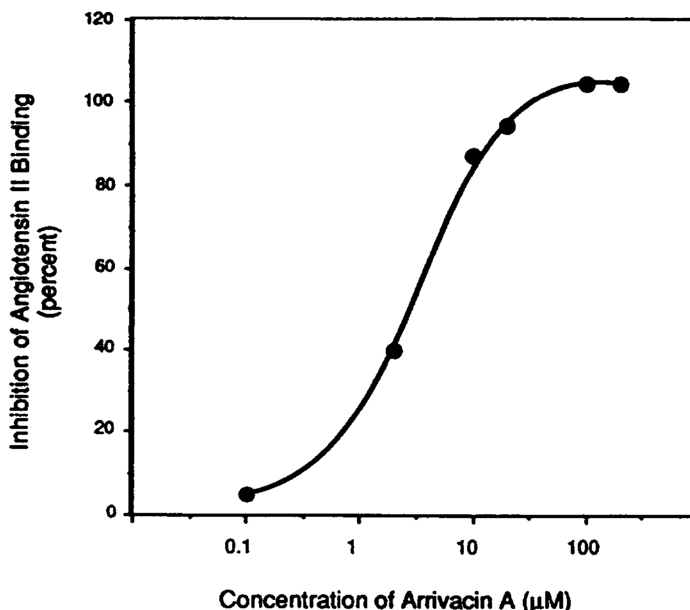


Figure 2. Inhibition of [^{125}I]angiotensin II receptor binding by arrivacin A (1). Arrivacin A was added to a standard reaction mixture as described in the Experimental section. Each point is the mean of three determinations. The data were analyzed by curve fitting (line) using the logistic function described by De Lean *et al.*[17]

EXPERIMENTAL

General Methods: Optical rotations were performed in MeOH relative to the D line of sodium using a Jasco DIP-360 digital polarimeter equipped with a constant temperature bath held at 25°C. Infrared spectra were recorded on a Nicolet Model 20 DXB FTIR Spectrometer. Ultraviolet spectra were recorded on a Beckman DU-7 spectrophotometer. ^{1}H and ^{13}C-NMR spectra were obtained using a Bruker WM360 operating at ambient temperature (29°C) and included ^{1}H COSY and ^{1}H/^{13}C correlation 2D NMR measurements, proton decouplings, difference NOE and ^{13}C edited spectra. All chemical shifts are reported with respect to TMS (δ0). Fast atom bombardment (FAB) was performed on a VG ZAB-SE-4F tandem double-focussing mass spectrometer (VG Analytical Ltd., Manchester, U.K.) operated at 10 kV ion-acceleration and equipped with a high-voltage (35kV) cesium ion gun. The matrix used was thioglycerol. Resolution for FAB exact mass measurements was 10000 (10% valley definition). Desorption chemical ionization (DCI) was conducted on a Finnigan 4610 quadrupole instrument scanned from m/z 60 to 700 in 1 second with a DCI probe ramp rate of 10 milleamps/sec. High resolution electron impact (EI) spectra were recorded on a VG 70-SE operated at 5000 resolution and employing 2 sec/decade scans from m/z 700 to 50. All solvents used were either HPLC or spectrophotometric grade.

Collection and Extraction Procedures: *Ambrosia psilostachya* was collected in Arizona, Pima County, Arrivaca Cienega on September 25, 1989 (BNT and SPM 5832). A herbarium specimen has been deposited at the University of Arizona, Tucson, Arizona. All plant material was air-dried, ground to 3mm particle size and stored

at 5° C prior to extraction. The milled aerial parts of *A. psilostachya* (4,300 g) were extracted exhaustively at room temperature with CH_2Cl_2 in a batch extractor. The extract was stripped of solvent to give 263 g of a black gum which was stored at 5° C before work-up.

Preparation of Receptors: Angiotensin II receptor was prepared from bovine adrenal cortex as described by Birabeau et al.[18] Briefly, cortices from about 100 g of fresh bovine adrenals were homogenized in 500 mL of 50 mM Tris·HCl, pH 7.4 at 4° C, containing 10 mM EDTA, 140 mM NaCl, 10 mM $MgCl_2$, 1 mM phenylmethylsulfonylfluoride (PMSF) and 0.1 mM p-chloromercuriphenylsulfonic acid (Buffer A). Large particulate material was removed by centrifuging 15 minutes at 900 x g. Membranes were removed from the supernatant by centrifuging 30 min at 30,000 x g and discarding the resultant supernatant. The pellet was resuspended in 500 mL of Buffer A and recollected by centrifuging 30 min at 30,000 x g. The supernatant was discarded. The pellet was then suspended in Buffer B (1 mL per gram cortices) which contained 50 mM Tris·HCl, pH 7.4, 0.32 mM sucrose, 25% glycerol (v/v), 0.1 mM PMSF, 0.01% dimethylsulfoxide (v/v), 50 mg/mL soybean trypsin inhibitor and 0.35 M NaCl.

This suspension was routinely about 10-fold more concentrated than required in the final binding assay. Scatchard analyses of isotope dilution experiments consistently indicated a K_D of 1 nM to 2 nM.

Assay of Angiotensin II Receptor Binding: Binding of [^{125}I]angiotensin II to adrenal receptors was measured by mixing ligand and receptor in 100 µl of 50mM Tris·HCl, pH 7.4, containing 140 mM NaCl, 1 mM EDTA and 200 µglmL BSA. Inhibition of ligand binding was accomplished by adding inhibitor to an assay mixture containing receptor (0.1 µg/mL of protein) such that the fractional saturation was 20% to 30% in a absence of inhibitor. After 20 min at 25° C, the solution was made 0.5 nM in [^{125}I]angiotensin II (2.2 Ci/mol) and incubated another 20 min at 25° C. Particulate receptor was collected by filtration through a glass fiber mat, using a cell harvester by Skatron, and bound ligand was measured with a gamma radiation counter (Beckman 5500B).

Isolation of Arrivacins A (1) and B (2): The crude CH_2Cl_2 extract (50 g) was chromatographed over silica gel (EM Reagents Kieselgel 60, 0.04 - 0.06 mm particle size, 500 g) using a solvent gradient from hexane through diethyl ether, ethyl acetate, and methanol. Fractions were monitored for activity in the AII receptor binding assay. The active fractions were combined (21 g) and further separated by reverse-phase C18 column chromatography (Whatman partisil-40 ODS-3, 300 g) using a solvent gradient of from 30% to 100% MeCN/H_2O. The active fraction (0.6 g) was purified by repeated reverse-phase HPLC (Beckman Ultraprep C18, 51mm x 150mm) using a 50% MeCN/H2O mobile phase to give the crude active arrivacins. Decolorization with activated charcoal in EtoAc gave arrivacin A (**1**, 85mg, 0.01% dry wt.) and arrivacin B (**2**, 18mg, 0.002% dry wt.) as amorphous white solids.

Arrivacin A (**1**): $[\alpha]_D^{25}$ -30.6° (c = 0.6, MeOH); FTIR (KBr) 3466, 2900, 1759, 1720, 1657, 1628, 1460, 1380, 1339, 1272, 1243, 1176, 1153, 1121, 1056, 1014, 983, 948, 906, 814, 756 cm^{-1}; UV (MeOH) 212 nm (ε 15,000); ^{1}H NMR Table 1; ^{13}C NMR Table 2; high resolution EIMS found m/z 514.2928, $C_{30}H_{42}O_7$ (M^+) requires m/z 514.2930, m/z 496.2833, $C_{30}H_{40}O_6$ (M^+ - H2O) requires 496.2825, m/z 478.3 for $C_{30}H_{30}O_6$, m/z 235.1683 for $C_{15}H_{23}O_2$, m/z 217.1589 for $C_{15}H_{21}O$.

Arrivacin B (2): $[\alpha]_D^{25}$ -46.8° (c = 1.0, MeOH); FTIR (KBr) 3504, 2925, 1762, 1748, 1650, 1625, 1455, 1408, 1384, 1330, 1312, 1277, 1240, 1173, 1153, 1111, 1081, 1055, 993, 981, 943, 908, 756 cm^{-1}; UV(MeOH) 214 nm (ε 18,400); ^1H NMR Table 1; ^{13}C NMR Table 2; high resolution FABMS found m/z 479.2796, $C_{30}H_{39}O_5$ $[(M + H)^+ - H_2O]$ requires 479.2797.

Isolation of coronopilin (3) and parthenin (4): The crude CH_2Cl_2 extract (2.3 g) was chromatographed over silica gel (EM Reagents Kieselgel 60, 0.04 - 0.06 mm particle size, 20 g) using a solvent gradient from hexane through diethyl ether, ethyl acetate, and methanol. The material eluted with diethyl ether (644 mg) was rechromatographed on silica gel using a solvent gradient from methylene chloride through 50% isopropanol/methylene chloride. The material eluted with 1% IPA/CH_2Cl_2 (242 mg) was repeatedly chromatographed on silica HPLC (Whatman M9 partisil, 50cm) using 7/3 Et_2O/EtOAc as a mobile phase to give coronopilin (**3**, 57 mg, 0.15% dry wt.) and parthenin (**4**, 12 mg, 0.03% dry wt.) as colorless powders.

Coronopilin (3): $[\alpha]_D^{25}$ +0.4° (c = 1.0, MeOH); FTIR (KBr) 3459, 2989-2861, 1740, 1657, 1409, 1392, 1344, 1278, 1259, 1238, 1168, 1130, 1119, 1070, 1062, 1033, 1015, 1103, 982, 973, 956, 946, 914, 824, 615 cm^{-1}; UV(MeOH) 215 nm (ε 10,000); ^1H NMR ($CDCl_3$) δ 1.10 (s, 3H, H_3-15), 1.17 (d, 3H, J = 7.6 Hz, H_3-14), 3.34 (m, 1H, H-7), 4.89 (d, 1H, J = 8.1 Hz, H-6), 5.57 (d, 1H, J = 2.4 Hz, H-13b), 6.20 (d, 1H, J = 2.8 Hz, H-13-a); ^{13}C NMR ($CDCl_3$) δ 14.6q (C-15), 17.3q (C-14), 27.6t (C-9), 30.1t (C-8), 32.0t (C-3), 32.7t (C-2), 42.5d (C-10), 44.6d (C-7), 58.8s (C-5), 79.6d (C-6), 84.7s (C-1), 121.4t (C-13), 141.0s (C-11), 170.6s (C-12), 218.0s (C-4); high resolution DCI, methane found m/z 265.1464, $C_{15}H_{20}O_4$, $(M + H)^+$ requires 265.1440.

Parthenin (4): $[\alpha]_D^{25}$ +10.1° (c = 1.0, MeOH); FTIR (KBr) 3440, 2984, 2963, 2947, 2927, 2877, 1747, 1721, 1658, 1636, 1477, 1450, 1404, 1386, 1339, 1326, 1274, 1245, 1205, 1163, 1118, 1095, 1071, 1063, 1022, 986, 979, 962, 945, 881, 819, 750, 689, 664, 650, 631, 615 cm^{-1}; UV (MeOH) 214 nm (ε 16,200); ^1H NMR ($CDCl_3$) δ 1.12 (d, 3H, J = 7.8 Hz, H_3-14), 1.28 (s, 3H, H_3-15), 1.68 (ddd, 1H, J = 14.0, 7.0, 3.5 Hz, H-9b), 1.84 (ddd, 1H, J = 14.0, 8.0, 3,5 Hz, H-8b), 2.15 (m, 1H, H-8a), 2.20 (m, 1H, H-9a), 2.21 (s, 1H, C1-OH), 2.31 (m, 1H, H-10), 3.49 (m, 1H, H-7), 5.00 (d, 1H, J = 7.8 Hz, H-6), 5.59 (d, 1H, J = 2.5 Hz, H-13b), 6.18 (d, 1H, J = 6.0 Hz, H-3), 6.27 (d,1H, J = 2.5 Hz, H-13a), 7.52 (d, 1H, J = 6.0 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 17.4q (C-14), 18.3q (C-15), 28.3t (C-9), 29.9t (C-8), 40.8d (C-10), 44.3d (C-7), 59.2s (C-5), 78.6d (C-6), 84.5s (C-1), 121.6t (C-13), 130.0d (C-3), 140.4s (C-11), 169.7d (C-2), 170.6s (C-12), 210.2s (C-4); high resolution DCI, methane found m/z 263, $C_{15}H_{18}O_4$, $(M + H)^+$ requires 263.1283.

Methanolysis of Arrivacin A (1): Arrivacin A (**1**, 15mg, 0.03 mMole) was stirred in 0.02M K_2CO_3/MeOH at room temp. for 18h. The reaction solvent was evaporated under a stream of nitrogen and the crude mixture was purified on reverse phase HPLC (Beckman ultraprep C18, 51mm x 150mm) using 50% MeCN/H2O as mobile phase to give the methyl ester **7** (1.0 mg, 10% theoretical). FTIR (KBr) 3600-3100, 3100-2800, 1722, 1626, 1439, 1383, 1280, 1243, 1197, 1174, 1136, 1089, 1058, 938, 909, 817 cm^{-1}; UV (MeOH) 214 nm; ^1H NMR Table 1; ^{13}C NMR Table 2; low resolution DCI, ammonia found m/z 284, $C_{16}H_{26}O_3$, $(M + NH_4)^+$.

Dehydration of Arrivacin A (**1**): Arrivacin A (**1**, 5mg, 0.01 mMole) was stirred at room temperature in a mixture of acetic anhydride (0.5 mL) and dry pyridine (0.5 mL) for 18h. The reagents were removed in vacuo and the crude product was purified on preparative reverse phase TLC (Whatman KC18F, 200 µM layer) using 50% MeCN/H_2O as the developing solvent to give **2** (2mg, 0.004 mMole, 40% theoretical) identical in all respects to the natural product.

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REFERENCES

1. Douglas, W. W. *The Pharmacological Basis of Therapeutics*, MacMillan Publishing Company (Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F., eds.), **1985**, 639-653.
2. Antonaccio, M. J. *Ann. Rev. Pharmacol. Toxicol.* **1982**, *22*, 57.
3. Yoshioka, H.; Mabry, T. J.; Timmermann, B. N. *Sesquiterpene Lactones, Chemistry, NMR and Plant Distribution*, University of Tokyo Press, **1973**.
4. Herz, W.; Hogenauer, G. *J. Org. Chem.* **1961**, *26*, 5011.
5. Geissman, T.A.; Griffin, S.; Wadell, T. G.; Chen, H. H. *Phytochemistry* **1969**, *8*, 145.
6. Miller, H. E.; Mabry, T. J.; Turner, B. L.; Payne, W. W. *Amer. J. Botany* **1968**, *55*, 316.
7. Miller, H. E.; Mabry, T. J. *J. Org. Chem.* **1967**, *32*, 2929.
8. Romo, J. P.; Nathan, J.; Siade, G. *Tetrahedron* **1966**, *22*, 1499.
9. Mabry, T. J.; Renold, W.; Miller, H.E.; Kagan, H. B. *J. Org. Chem.* **1966**, *31*, 681.
10. Mabry, T. J.; Miller, H. E..; Kagan, H. B. Renold, W. *Tetrahedron* **1966**, *22*, 1139.
11. Mabry, T. J.; Kagan, H. B.; Miller, H. E. *Tetrahedron* **1966**, *22*, 1943.
12. Kagan, H. B.; Miller, H. E.; Renold, W.; Lakshmikanthan, M. V.; Tether, L. R.; Herz, W.; Mabry, T. J. *J. Org. Chem.* **1966**, *31*, 1629.
13. Yoshioka, H.; Mabry, T. J.; Miller, H. E. *J. Chem. Soc. Chem. Comm.* **1968**, 1679.
14. Herz, W.; Gage, D.; Kumar, N. *Phytochemistry* **1981**, *20*(7), 1601.
15. Fischer, N. H.; Oliver, E. J.; Fischer, H. D. *Fortschritte der Chemie Organischer Naturstoffe* **1979**, *38*, 223-228.
16. Balza, F.; Towers, G. H. N. *Phytochemistry* **1988**, 27, 1421.
17. De Lean, A.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.
18. Birabeau, M. A.; Capponi, A. M.; Vallotton, M. B. *Mol. and Cell. Endocrinology* **1984**, *37*, 181.