Phytochemistry, Vol. 26, No. 4, pp. 1059–1063, 1987.
Printed in Great Britain.

0031-9422/87 $3.00+0.00

SESQUITERPENE LACTONES AND A SESQUITERPENE DIOL FROM JAMAICAN *AMBROSIA PERUVIANA*

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(*Received* 10 *April* 1986)

Key Word Index—*Ambrosia peruviana*; Compositae; wild tansy; pseudoguaianolides; alloaromadendrane diol.

Abstract—A new sesquiterpene diol and four pseudoguaianolides have been isolated from the aerial part of a Jamaican collection of *Ambrosia peruviana*. The structures have been identified as alloaromadendrane-4β,10α-diol, psilostachyins C and B, ambrosin and damsin, respectively, by chemical and spectroscopic means.

INTRODUCTION

Ambrosia peruviana Willd., wild tansy, is a slightly aromatic shrubby herb found mainly in the Caribbean [1]. Its tendency to grow in isolated clusters amid other vegetation suggested that the plant may possess allelopathic activity of the type reported for other members of the genus *Ambrosia* [2, 3]. *Ambrosia psilostachya* and *A. cumanensis* both exhibit inhibitory activity against the growth of a variety of plant species [4–6]. Mixed sesquiterpenes isolated from ragweed, *A. artemisiifolia*, were very inhibitory to onion, oat and ryegrass [7]. Previously, a collection of *A. peruviana* from Mexico was reported to contain peruvin (**1**) [8] and peruvinin (**2**) [9]. By contrast, a collection from Puerto Rico gave tetrahydroambrosin (**3**) and psilostachyin C (**4**) [10, 11]. We now report the results of our efforts to locate and identify potential allelopathic compounds from a Jamaican specimen of *A. peruviana*.

RESULTS AND DISCUSSION

Sequential extraction of dried, ground stem and leaf material of *A. peruviana* with hexane, ethyl acetate and methanol, respectively, afforded three fractions. Of these, only the ethyl acetate extract showed activity in our seed germination bioassay. Five sesquiterpenes—four lactones of the psuedoguaianolide type (**4–7**) and one diol (**8**)—were characterized from this ethyl acetate extract. Compounds **4–7** were identified by analysis of the mp's, IR, mass spectra, ^{1}H NMR and ^{13}C NMR data and subsequent comparison of these observed physical characteristics with previously published data for psilostachyins C (**4**) and B (**5**) [10, 12–15], ambrosin (**6**) and damsin (**7**) [16–21]. While compounds **4–7** are common among *Ambrosia* species, this is the first report on the isolation of compounds **5–7** in *A. peruviana*.

The structure of **8** ($C_{15}H_{26}O_2$), mp 112–113°($[\alpha]_D$ +7°) was clearly unrelated to the others, as was evident from its chromatographic behaviour. The compound had a lower R_f on silica gel plates, and stained pink at room temperature with an aqueous sulphuric acid spray [2, 3]. In addition, it had no UV-absorbing chromophore and showed hydroxyl absorption (3500 cm^{-1}) in the IR spectrum. The ^{1}H NMR and ^{13}C NMR spectral data of **8** compare favourably with those of other sesquiterpene diols. In particular, signals for tertiary methyl groups at δ1.02, 1.03, 1.18 and 1.33, two cyclopropyl protons at 0.00 (1H, *t*, $J_{5,6} = J_{6,7} = 9.7$ Hz) and 0.62 (1H, *ddd*, $J_{6,7} = 9.7$ Hz, $J_{7,8\alpha} = 11.6$ Hz, $J_{7,8\beta} = 5.8$ Hz) and the carbinol carbons at 74.33 and 82.14 suggested a type of 4,10-aromadendrane diol [22–25]. The complete structure and stereochemistry of **8** were assigned on the evidence which follows.

Since the relative intensity of the molecular ion (*m/z* 238) of **8** was only 1% in the mass spectrum, the diol moiety was confirmed by silylation of **8** to the di-*O*-TMSi derivative (**9**). Two singlets (9H each) at δ0.08 and 0.12 in the ^{1}H NMR spectrum of **9** established the presence of the two trimethylsilyl groups. The molecular ion (*m/z* 382) in the mass spectrum of **9** was also consistent with the formulation of **8** as a diol. Compound **8** could not be the known diols **10–13** derived from spathulenol [22] because despite their similarity, they differed from **8** not only in their NMR spectral data, but also in melting points and molecular rotation [22–25]. The difference between **8** and these aromadendrane diols must therefore be associated with the stereochemistry of the ring juncture carbon atoms (C-1, C-5, C-6 and C-7). This difference was resolved by comparison with known chemical shift data and shift parameters [26] and by using selective homonuclear and heteronuclear decoupling to confirm our assignments.

A *trans*-orientation of the cyclopropane ring was discounted primarily because of the ^{13}C NMR spectral data of the geminal dimethyl group on the cyclopropane ring. The chemical shifts (16.42 and 28.55) of these methyl groups correlate well with those previously assigned to the corresponding methyl groups in **10–13** [22, 23]. Similarly, examples of geminal dimethyl groups attached to *cis*-oriented cyclopropane rings differed appreciably from those expected for geminal dimethyl groups attached to a cyclopropane ring to which substitutents are attached in a

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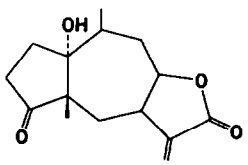

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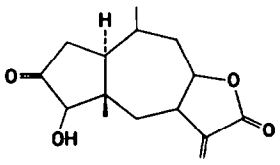

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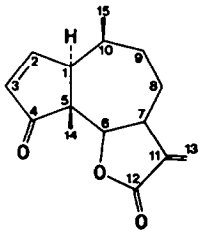

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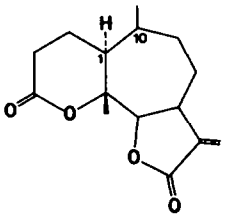
6 2,3-dihydro-3

7 2,3,11,13-tetrahydro-3



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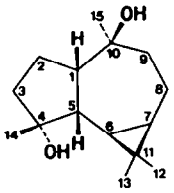
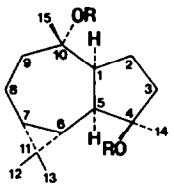
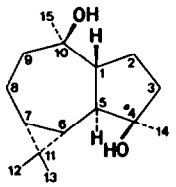
5 1,10-dehydro-4





8 R=H

9 R=TMSi



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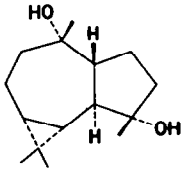

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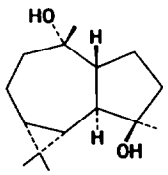

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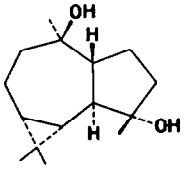

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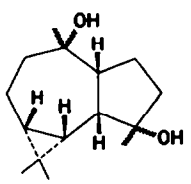
Table 1. ^{13}CNMR ($CDCl_3$) spectral data of sesquiterpene diols

C	8*	10†	11†	12†	13†
1	54.0	54.5	52.5	56.4	56.2
2	25.1	23.7	23.7	24.3	23.7
3	37.4	40.3	39.6	41.4	41.0
4	82.1	80.1	80.5	80.3	80.1
5	47.8	47.5	45.8	47.2	48.2
6	25.3	25.0	24.9	30.1	28.3
7	28.8	26.2	27.0	26.9	26.5
8	18.7	20.3	19.1	19.2	20.0
9	37.9	44.2	42.4	42.8	44.3
10	74.3	75.3	72.8	71.8	74.8
11	18.6	18.9	20.1	20.9	19.4
12	28.5	28.8	29.0	28.7	28.5
13	16.4	16.4	16.4	16.4	16.3
14	25.6	25.6	25.8	25.1	24.3
15	32.2	19.7	31.2	30.5	20.0

*Measured at 75.4 MHz.

†Taken from refs. [22] and [23].



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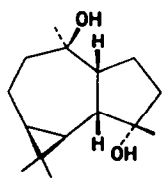

15

trans-orientation [27, 28]. In addition, the observed proton–proton coupling constants ($J_{6,7}$ = 9.7 Hz) of the protons attached to the cyclopropane ring supports a *cis*-fusion. A β-orientation of H-5, as shown in **14**, was excluded since it would lead to a ring conformation with a dihedral angle of 60° between H-5 and H-6. Such an angle would produce a much smaller coupling constant than the 9.7 Hz observed for these protons. Further studies of molecular models indicate that the relative orientation of H-5 and H-6 shown in **8** leads to an almost *trans*-antiperiplanar relationship between these protons, result-

ing in the observed coupling constant of 9.7 Hz, similar to values shown in the spectra of compounds **10–13** [22–25]. Since the stereochemical relationships at C-5, C-6 and C-7 are retained, this new diol must possess the alloaromdendrane skeleton with the α-orientation of H-1 and the *cis*-ring juncture as shown in **8**.

The stereochemistry of the hydroxyl groups at C-4 and C-10 was also assigned by correlating features of molecular models with data from the NMR spectra. In the most acceptable conformation of a *cis*-fused seven-membered ring the close approach of a β-oriented hydroxyl group at C-10 to H-6 and H-7 would be expected to deshield these protons [29]. This is not observed. Instead, the methyl group (C-15) appears at a slightly lower field (32.16) in the ^{13}C NMR spectrum, reflecting the two δ-*gauche* interactions with C-6 and C-7 [26], and is therefore in a β-orientation. On the other hand, the assignment of δ25.6 to the C-14 methyl group is consistent with the structure (**8**). This signal does not differ greatly from the corresponding signals for compounds **10–13** regardless of the stereochemistry at C-4, because models show that a change in the stereochemistry at C-1 does not significantly affect the spatial relationship between C-14, C-4, C-5 and C-7. The answer to the stereochemistry at C-4, therefore, is found in the comparison of the chemical shifts of C-6 in the ^{13}C NMR spectra of the pair of C-4 epimers (**10, 11**) and (**12, 13**). When C-14 is in an α-orientation, as in **10** and **11**, C-6 appears at δ25.0 and 24.9, respectively. In contrast, the downfield signals of C-6 at δ30.1 and 28.3 in the spectra of **12** and **13** coincide with a β-orientation of this methyl group. Hence, a value of 25.28 for C-6 in the spectrum of **8** establishes an α-orientation of C-14. Consequently, the hydroxyl group at C-4 is in a β-orientation. This assignment is supported by the lowfield position for H-1 at δ2.48 in the ^{1}H NMR spectrum of **8**. In **12** and **13** where the C-4 hydroxyl group and H-1 are on opposite sides of the five-membered ring, no proton appears below δ2.0 in the ^{1}H NMR spectra. However, in **10** and **11** where the C-4 hydroxyl and H-1 are in a *cis*-1,3-pseudodiaxial relationship across the five-membered ring, H-1 experiences a paramagnetic shift to δ2.2 [29]. Thus, the position of H-1 at δ2.48 in the spectrum of **8** adds to the validity of the stereochemical assignment shown in **8**. Compound **8** is thus (+)-alloaromadendrane-4β,10α-diol.

The isolation of **8** from *A. peruviana* is the first reported characterization of a sesquiterpene diol from *Ambrosia* species. It is of significance for two reasons. Firstly, it relates to a compound isolated from the marine soft coral, *Sinularia mayi*, the structure of which was not determined, but to which was assigned only a tentative structure [23]. Direct comparison of **8** with the metabolite from *S. mayi* showed that these compounds are identical in all respects except in their rotation ($[\alpha]_D$ + 7° and − 10°, respectively), and therefore establishes structure **15** for this marine metabolite. Compounds **8** and **15** are therefore antipodal alloaromadendrane sesquiterpene diols. They illustrate the generally observed antipodal relationship between sesquiterpenes derived from terrestrial and marine organisms [30, 31]. Secondly, the carbon skeleton of **8** with its methyl group at C-4, instead of at the ring juncture (C-5), is structurally more directly related to guaianolides found in *Artemisia* species than the pseudoguaianolides of *Ambrosia* species. It therefore offers support to the relation of the genus *Ambrosia* to *Artemisia*, previously observed in the *Ambrosia cumanensis* complex of Mexico [32].

The biological activity of the crude extracts and purified components consisted of simple tests of monitoring the germination of seeds and the growth of seedlings of lettuce and cress. These seeds were used because of the high percentage of germination in the controls and the speed with which they germinate. Results in these bioassays were usually obtained in 24 hr. In view of the very low solubility of the crude extract and the purified compounds in water, the filter paper on which the seeds were to be grown was first impregnated with the substance. A 90% inhibition of lettuce seed germination was shown by the ethyl acetate crude extract at an application of 1.4 μg/mm^2 of filter paper. After initial preparative TLC only fractions containing 'active' components were further investigated. Upon final purification ambrosin (**6**) and psilostachyin **B** (**5**) were most active in preventing germination and root and shoot growth in lettuce and cress seedlings. At 0.3 μg/mm^2, complete inhibition of growth was observed even after 72 hr. Psilostachyin C (**4**) and damsin (**7**) exhibited both stimulatory and inhibitory effects on seedling growth at medium (0.14 μg/mm^2) and low (0.07/mm^2) concentrations, respectively. Compound **8** caused marginal reduction in the growth of cress seeds but stimulated root and shoot growth in lettuce at low concentrations (0.07 μg/mm^2). Further assays for antifungal activity were performed by direct bioautography on TLC using *Cladosporium herbarium* as test organism [33]. *C. herbarium* was unaffected by compounds **4–6**. However, damsin (**7**) and, to a much greater degree, (+)-

Table 2. Growth of cress and lettuce root and shoot in the presence of sesquiterpenes (μg/mm^2) from *A. peruviana* expressed as a percentage of control

	Cress						Lettuce					
	Root			Shoot			Root			Shoot		
Compound	0.28*	0.14	0.07	0.28	0.14	0.07	0.28	0.14	0.07	0.28	0.14	0.07
4	38	49	48	60	86	82	66	101	118	74	94	142
5	0	30	41	0	47	82	0	34	76	0	31	85
6	0	11	37	0	11	64	0	0	32	0	0	30
7	43	46	75	50	89	88	14	68	104	11	61	106
8	10	26	64	20	35	67	30	107	135	47	149	158

*Expressed as μg of compound/mm^2 of filter paper.

alloaromandendrane-4β,10α-diol (**8**) were very effective inhibitors to the growth of this fungus.

EXPERIMENTAL

All mps are uncorr. ^{1}H NMR were recorded at 300 MHz using TMS as internal standard and ^{13}C NMR were recorded at 75.4 MHz in $CDCl_3$. IR spectra were recorded in $CHCl_3$ soln. EIMS were determined at 70 eV, direct inlet. Prep. TLC was performed using silica gel plates (Merck, 1 mm and 0.5 mm) with mixtures of $CHCl_3$ and MeOH as eluant. Plates were developed with I_2 vapour or by spraying with 50% aq. H_2SO_4. Plant material was collected in January 1984 at St. Ann, Jamaica and was identified by Dr. G. R. Proctor. Samples and voucher number were deposited at the herbarium of the Institute of Jamaica.

Extraction and isolation. Ground, air-dried stem and leaf material was extracted by sequential, cold percolation of solvents (*n*-hexane followed by EtOAc, and MeOH, respectively) through the plant grounds contained in a column. By this method, extraction of 102 g of plant material afforded 1.4 g crude residue from elution with hexane, 3.1 g from elution with EtOAc and 5.3 g from elution with MeOH. Purification of the EtOAc crude (243 mg) by successive prep. TLC yielded compound **8**, 12.8 mg (0.010%); ambrosin (**6**), 4.7 mg (0.006%); damsin (**7**), 16.3 mg (0.02%); psilostachyin B (**5**), 11.0 mg (0.013%); and psilostachyin C (**4**), 52.1 mg (0.076%). The relative purity of the compounds was assessed by HPLC (Waters) using a 5 μm C_{18} Novapak column with MeCN–H_2O (3:2) as solvent system (detection at 210 nm).

The identity of the known sesquiterpene lactones, **4**–**7**, was established by comparison of mps, ^{1}H NMR, ^{13}C NMR, MS and IR with previously reported data.

Alloromadendrane-4β,10α-diol (**8**). Colourless prisms, $C_{15}H_{26}O_2$, mp 112–113°, $[\alpha]^{25}+7°$ ($CHCl_3$, *c* 0.45). IR ν_{max} cm^{-1}: 3600. ^{1}H NMR: δ2.47 (1H, *m*, H-1), 185 (1H, *dd*, H-2), 1.75–1.55 (H, *m*, CH_2), 1.33 (3H, *s*, C-10 Me), 1.19 (3H, *s*, C-4 Me), 1.02 and 1.03 (3H, *s*, geminal Me), 0.62 (1H, *ddd*, $J_{6,7}$ = 9.7 Hz, $J_{7,8\alpha}$ = 11.6 Hz, $J_{7,8\beta}$ = 5.8 Hz, H-7), 0.00 (1H, *t*, $J_{5,6}$ = $J_{6,7}$ = 9.7 Hz, H-6). ^{13}C NMR ($CDCl_3$): see Table 1; high-resolution EIMS *m/z* (re. int.): 238.1946 $[M]^+$ (1) (calc. for $C_{15}H_{26}O_2$: 238.1933), 220 (16), 205 (23), 162 (100), 147 (54), 119 (75). Preparation of the *O*-silyl derivative was accomplished by a standard method using Pierce Tri-sil [34]. ^{1}H NMR ($CDCl_3$): δ1.31 (3H, *s*, C-10 Me), 1.19 (3H, *s*, C-4 Me), 1.01 (6H, *s*, geminal Me2), 0.12 (9H, *s*, TMSi-O), 0.08 (9H, *s*, TMSi-O). EIMS *m/z* (rel. int.): 382 $[M]^+$ (4).

Tests for biological activity. Preliminary tests for inhibition of lettuce seed germination were conducted as follows: crude residues from elution with hexane, EtOAc and MeOH were dissolved in Me_2CO to a concn of 5 mg/ml from which 500 μl or 250 μl was applied to filter paper (Whatman No. 1, 4.25 cm) in glass Petri dishes (60 × 15 mm). Each filter paper was dried for several hr by placing the dish in a vacuum dessicator. Lettuce seeds (30, Great Lakes, Lot No. 659 Ws) were placed on the filter paper. Deionized H_2O (2 ml) was then added before sealing with parafilm. Dishes were stored in the dark and the number of seeds germinated was recorded at 24 hr intervals. Pure compounds were assayed in a manner similar to that described for the crude EtOAc residue. Compounds were dissolved in CH_2Cl_2 (1 mg/ml) and applied to filter paper in aliquots of 500, 200 and 100 μl before removal of solvent *in vacuo*. A total of 20 seeds of each type (lettuce and cress; cress variety from Harris, seed lot No. 396–297) was used. Seeds of each variety were placed on the dried filter paper and H_2O (2 ml) was added. The dishes were kept in the dark. The number of seeds germinated was recorded at 24 hr intervals. At 72 hr, root and shoot growth was measured. Tests for antifungal activity were conducted by applying compounds **4**–**8** in the amounts of 25, 10 and 5 μg to a silica gel plate and then spraying the plate with a suspension of spores of *Cladosporium herbarium* contained in Homans media (30 ml) supplemented with 1.5 g glucose. Spores were allowed to grow for 2–3 days before inhibition was recorded.

Acknowledgements—The authors are grateful to Dr. G. R. Proctor for his help in identifying the plant material, Prof. C. Djerassi and Dr. C. Beechan for a sample of the antipodal diol from *S. mayi*, D. Hirano for MS measurements, and to J. Bourell for assistance in high-field NMR operations.

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