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AMBROSANOLIDES AND SECOAMBROSANOLIDES FROM *AMBROSIA TENUIFOLIA*

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Key Word Index—*Ambrosia tenuifolia*; Ambrosiinae; Heliantheae; Compositae; sesquiterpene lactones; ambrosanolides; secoambrosanolides.

Abstract—Populations of *Ambrosia tenuifolia* from four regions of North Central Argentina differed substantially in sesquiterpene lactone content. Lactones and lactone derivatives isolated were peruvin, ambrosic acid, cumanin, psilostachyin, psilostachyins B and C, dihydropsilostachyin C, altamisin and altamisic acid.

INTRODUCTION

A number of years ago one of us [1] briefly reported *inter alia* the isolation of the secoambrosanolide psilostachyin (**6**) from *Ambrosia tenuifolia* Spreng., a South American species which extends from Central Argentina to Uruguay and Paraguay [2]. Since phytochemical studies of *Ambrosia* and related species have shown that sesquiterpene lactones are useful taxonomic characters at various levels within subtribe Ambrosiinae of Heliantheae [3] we now report the results of a thorough examination of four Argentinian populations of this species.

RESULTS AND DISCUSSION

A population of *A. tenuifolia* from Entre Rios Province furnished the relatively uncommon C-8 lactonized ambrosanolide peruvin (**1**), the rare ambrosic acid (**2a**), another C-8 lactonized ambrosanolide cumanin (**3**) and a relatively small amount of the secoambrosanolide psilostachyin C (**4**) admixed with its 11,13-dihydro derivative **5**. A population from Corrientes was similar chemically, with ambrosic acid as the main constituent, although **4** and **5** were not isolated. A third collection from Tucumán Province afforded **3** as the main sesquiterpene lactone constituent and smaller amounts of **4** and psilostachyin (**6**) whereas a collection from Córdoba Province gave only secoambrosanolides, namely **4**, **6**, psilostachyin B (**7**), altamisin (**8b**) and its previously unknown parent altamisic acid (**8a**). The structure of **8a** was deduced by comparing its ^{1}H NMR and ^{13}C NMR spectra with those of **8b** [4, 5] and conversion to a pyrazoline (Tables 1 and 2). The previously unreported high resolution ^{1}H NMR spectra of **1–3** and the ^{13}C NMR spectra of **1** and **3** are also listed in the Tables.

Peruvin (**1**) was originally found [6] in a collection of *A. peruviana* from Hidalgo, Mexico, and has since been reported only from a Zacatecas collection of *A confertiflora* [7] and Russian *A. artemisiifolia* [8]. Ambrosic acid (**2a**) was initially synthesized from peruvin [6] and was subsequently discovered as a natural product in Japanese *A. artemisiifolia* [9]. Cumanin (**3**) has been identified only in a few populations of *A. psilostachya* [10–13], *A. cumanensis* [10, 11]* and *A. artemisiifolia* [8] whereas psilostachyin (**6**), psilostachyin B (**7**) and psilostachyin C (**4**) are more widely distributed in *Ambrosia* species [3]. Altamisin has so far been isolated only from an El Salvador collection of *A. cumanensis* [4].

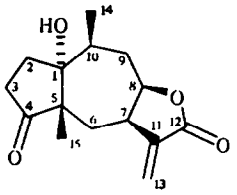
The sesquiterpene lactone chemistry of our *A. tenuifolia* collections seems to be in line with the proposal that the species, together with *A. peruviana*, represents the Southern Hemisphere extension of the recently derived specialized North American *A. cumanensis–A. psilostachya–A. artemisiifolia* complex [2]. Further sampling is necessary to inquire into the existence of chemotypes and to determine whether the chemistry correlates with differences in morphology, if any, and chromosome count information which does not seem to exist currently.

EXPERIMENTAL

Isolation of Ambrosia tenuifolia *constituents.* (A) Entre Ríos collection, collected and identified by Ing. Arg. J. J. Jozami of IPNAIS, Santa Fe in October 1983 in Bajada Grande, Paraná, Entre Rios Prov., Argentina. Above ground material, 5 kg, was extracted with 5 × 12 kg $CHCl_3$ and worked up in the usual fashion [14] to give 95 g of crude gum, a 20 g portion of which was dissolved in hot C_6H_6. On cooling there precipitated 274 mg of peruvin (**1**). The C_6H_6-soluble material was chromatographed over 450 g of silica gel (70–270 mesh), 50 ml fractions being collected in the following order: C_6H_6, C_6H_6–$CHCl_3$ (1:1), $CHCl_3$–MeOH (99:1, 49:1 and 19:1). Fr. 18–19 gave 380 mg of peruvin, fr. 21 gave 90 mg of ambrosic acid (**2a**). Rechromatography of fr. 20–23 gave 180 mg of cumanin (**3**). Chromatography of the remaining 75 g of gum and rechromatography of the fractions of interest gave in addition to **1**, **2a** and **3**, 232 mg of psilostachyin C (**4**) mixed with about 10% of 11,13-dihydropsilostachyin C (**5**) (NMR analysis).

(B) Tucumán collection, collected by Dr. C. Catalán on November 24, 1983, in Montaegudo, Simoca, Tucumán Prov. and identified by Ing. Agr. P. R. Legname (voucher #9074 on

*In ref. [10] the collection of *A. psilostachya* was misidentified as *A. cumanensis*, see ref. [11].



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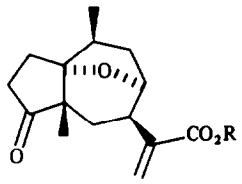

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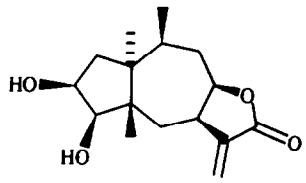
2b R = Et



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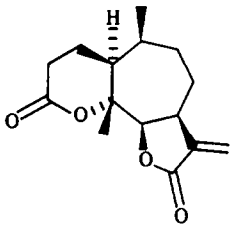
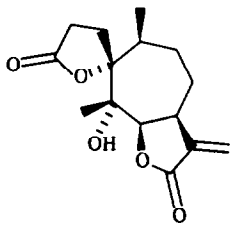


4
5 11,13-dihydro



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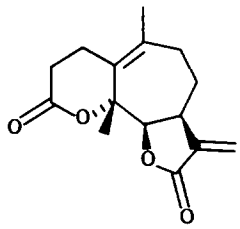

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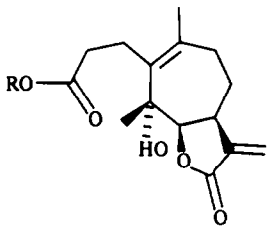

8a R = H
8b R = Et

deposit in Fundación Miguel Lillo, Tucuman). Above ground material (1.3 kg) was extracted three times with 7 kg $CHCl_3$ each. The residue from the first extraction (37% of total residue) was worked up in the usual fashion to give 19 g of crude gum which was taken up in hot C_6H_6–EtOAc. Cooling and recrystallization of the ppt from MeOH–$CHCl_3$ afforded 284 mg of psilostachyin (**6**). Chromatography of the soluble material over 450 g silica gel (60 mesh), 125 mg fractions being collected with $CHCl_3$ and $CHCl_3$–MeOH (99 : 1 and 49 : 1), gave in fr. 15–22 877 mg of **6**, in fr. 24–26 950 mg of a mixture of **4** and **6** and in fr. 29–31 1.99 g of **3**. Rechromatography of the mixture gave 712 mg **6** and 31 mg of **4**.

(C) Corrientes collection collected and identified by Dr. A. T. Ricciardi, Universidad Nacional del Noreste, Corrientes, in March of 1984 in the city of Corrientes, Corrientes Prov. Above ground material (1.5 kg) was extracted × 3 with 8 l. $CHCl_3$. The residue from the first extraction (50% of total residue) was worked up in the usual fashion to give 22.3 g of crude gum which was chromatographed over silica gel (60 mesh), 125 ml fractions being collected with $CHCl_3$, $CHCl_3$–MeOH (19 : 1, 9 : 1 and 4 : 1). Trituration of the various fractions with Et_2O afforded from fr. 2 ($CHCl_3$–MeOH, 19 : 1) 162 mg of **1**, from fr. 3 and 4 ($CHCl_3$–MeOH, 19 : 1) 781 mg of **2**, and from fr. 5 ($CHCl_3$–MeOH, 19 : 1) 65 mg of **3**. The mother liquors afforded an additional 375 mg of **2**.

(D) Córdoba collection (voucher no. 3035 on deposit in Museo Botanico, Córdoba, collected in Ascochinga, Dept. Colon, Córdoba Prov. in February 1984 and identified by Dr. Luis Ariza Espinar). Above ground material (1.88 kg) was extracted × 3 with 8 l. $CHCl_3$ each. The residue from the first extraction (43% of total residue) was worked up in the usual fashion to give 21.6 g of crude gum which was dissolved in hot EtOH. On cooling there precipitated 3.50 g of lactone mixture A. Evaporation of the filtrate and trituration of the residue with Et_2O–C_6H_6 afforded 1.06 g of lactone mixture B and, on prolonged standing, 2.65 g of lactone mixture C as well as a filtrate D. Chromatography of mixture A (37 g of silica gel, eluent $CHCl_3$, 20 ml fractions) gave in fr. 1–3 831 mg of a 1 : 1 mixture of **4** and **6** and in fr. 4–10 562 mg of **4**. Lactone mixture B was a 1 : 1 mixture of **4** and **6**. Mixture C was taken up in hot EtOH; cooling afforded a mixture of three lactones (997 mg) which was chromatographed over 30 g of silica gel (eluents $CHCl_3$ and $CHCl_3$–MeOH, 99 : 1). Fr. 1–4 (289 mg) were mainly **4** and fr. 5–8 (621 mg) were pure **6**. The residue (1.6 g) from the EtOH mother liquor of C was chromatographed (32 g silica gel, $CHCl_3$ and $CHCl_3$ with increasing amounts of MeOH). Fr. 1–14 ($CHCl_3$–MeOH, 199 : 1) afforded 789 mg of psilostachyin B (**7**). Fr. 29 ($CHCl_3$–MeOH, 99 : 1) eluted 62 mg of **6** and fr. 37 ($CHCl_3$–MeOH, 9 : 1) eluted 10 mg of altamisic acid (**8a**). More of **8a** was obtained by evaporating filtrate D and chromatographing the residue (250 g silica gel,

Table 1. ^{1}H NMR spectra (270 MHz, $CDCl_3$)

H	1†	2	3‡	7	8a
1	—	—	0.55 *m*	—	—
2a	2.42 *m*	1.74 *ddd*	1.40 *dd*	2.3–2.7 *c*	2.78 *ddd*
2b	1.69 *m*	2.3–2.5 *c*	1.50 *ddd*		2.3–2.6 *c*
3	2.46 *m*		3.59 *dt* (4.21)		
4	—	—	2.95 *d* (3.51)	—	—
6a	2.56 *dd*	1.51 *br t*	1.65 *dd*	4.81 *d*	4.74 *d*
6b	1.47 *dd*	2.13 *br dd*	1.13 *br t*	—	—
7	3.84 *ddddd* (3.62)	2.94 *br dt*	2.30 *ddddd* (3.14)	3.36 *ddddd*	3.53 *ddddd*
8	4.94 *ddd* (4.48)	4.36 *m*	3.57 *ddd* (4.68)	2.12 *ddt*, 1.88 *dddd*	2.16 *m*, 1.78 *m*
9a	2.20 *m*	2.22 *m*	1.76 *ddd*	2.3–2.7 *c*	2.3–2.6 *c*
9b	1.87 *m*	1.36 *m*	1.29 *m*		
10	2.05 *m*	2.22 *m*	1.29 *m*	—	—
13a	6.23 *d* (6.19)	6.42 *br*	6.20 *d* (6.27)	6.26 *d*	6.22 *d*
13b	5.66 *d* (5.07)	5.65 *br d*	4.97 *d* (5.62)	5.55 *d*	5.52 *d*
14*	1.22 *d* (0.59)	1.26 *d*	0.67 *d* (1.04)	1.77 *br*	1.74 *br*
15*	1.08 (0.49)	1.08	0.72 (0.98)	1.53	1.21

J (Hz), compound 1: 6a,6b = 16; 6a,7 = 5.5; 6b,7 = 12; 7,8 = 8; 7,13a = 3; 7,13b = 2.5; 8,9a = 12.5; 8,9b = 3; 10,14 = 7. Compound 2: 2a,3a = 8; 2a,3b = 3; 6a,6b = 6a,7 = 14; 6b,7 = 5.5; 7,8 = 5; 8,9a = 6.5; 10,14 = 7. Compound 3: 1,2a = 7; 1,2b very small; 2a,2b = 13; 2a,3 = 8; 2b,3 = 5; 3,4 = 10,14 = 7.5; 6a,6b = 15; 6a,7 = 4; 6b,7 = 15; 7,8 = 8; 7,13a = 8,9a = 3; 7,13b = 2.5; 8,9b small; 9a,9b = 15. Compound 7,8a: 6,7 = 9; 7,8a = 5.5; 7,8b = 12, 7,13a = 3.5; 7,13b = 3; 8a,8b = 14; 8a,9a = 4 or 5.5, 8a,9b = 5.5 or 4; 8b,9a = 10 or 5, 8b,9b = 5 or 10; in 8a, 2a,2b = 14.5; 2a,3a = 10; 2a,3b = 5.

*Intensity 3 protons.

†Figures in parentheses for C_6D_6 solution.

‡In C_6D_6 plus H_2O; figures in parentheses for $CDCl_3$ solution.

Table 2. ^{13}C NMR spectra ($CDCl_3$)

C	1*	3*	7†	8a†	8b†	9†
1	83.77 *s*	41.28 *d*	133.0 *s*	133.8 *s*‡	134.0 *s*‡	133.8 *s*‡
2	32.66 *t*	40.81 *t*	25.0 *t*‡	25.4 *t*	25.6 *t*	25.5 *t*
3	35.62 *t*	68.35 *d*	34.6 *t*	34.0 *t*§	34.0 *t*§	33.5 *t*
4	216.25 *s*	77.13 *d*	170.5 *s*	179.0 *s*	173.7 *s*	174.1 *s*
5	54.79 *s*	44.14 *s*	86.7 *s*	76.9 *s*	77.9 *s*	75.3 *s*
6	31.56 *t*	35.04 *t*	83.2 *d*	85.7 *d*	85.7 *d*	87.0 *d*
7	38.89 *d*	37.71 *d*	41.3 *d*	41.7 *d*	41.7 *d*	43.3 *d*
8	80.71 *d*	80.06 *d*	29.7 *t*	34.5 *t*§	34.8 *t*§	34.3 *t*
9	36.69 *t*	36.43 *t*	25.8 *t*‡	25.4 *t*	25.6 *t*	25.5 *t*
10	41.14 *d*	30.13 *d*	125.9 *s*	132.5 *s*‡	132.3 *s*‡	133.6 *s*‡
11	141.29 *s*	139.84 *s*	138.3 *s*	138.4 *s*	138.5 *s*	99.0 *s*
12	170.44 *s*	169.83 *s*	169.5 *s*	170.2 *s*	170.1 *s*	173.0 *s*
13	122.50 *t*	122.92 *t*	120.3 *t*	120.1 *t*	120.0 *t*	22.6
14	18.79 *q*	16.69 *q*	23.5 *q*	24.4 *q*	24.5 *q*	26.5 *q*
15	22.71 *q*	17.77 *q*	22.8 *q*	22.6 *q*	22.6 *q*	22.7 *q*
16					59.9 *q*	51.4 *t*
17					14.08 *q*	78.5 *t*

*Run at 67.89 MHz using DEPT sequence.

†Run at 20.15 MHz using DEPT sequence.

‡§Assignments with the same sign in the same column may be interchanged.

125 ml fractions, eluents $CHCl_3$ and $CHCl_3$–MeOH, 199:1, 49:1, 24:1, 23:2, 4:1). Rechromatography of fr. 2–6 eluted with $CHCl_3$ gave 46 mg of altamisin (**8b**); fr. 16 and 17 ($CHCl_3$–MeOH, 199:1) contained a lactone mixture, primarily 4 and 6. Extensive rechromatography of the material from fr. 20–28 ($CHCl_3$–MeOH, 23:2 and 4:1) and trituration with hot Et_2O eventually furnished 160 mg of **8b**.

Characterization of products. Peruvin (1), mp unsharp (dec.) lit. mp 191–193° [6]; UV λ_{max} 212 nm; IR ν_{max} cm^{-1}: 3449, 1739, 1723, and 1652; MS *m/z* (rel. int.): 254 (45.2), 246 (21.9), 138 (100).

Chemical shifts and coupling constants in the 270 MHz ^{1}H NMR spectrum (Table 1) compared with the 60 MHz data given in [6]; ^{13}C NMR spectrum in Table 2. Reaction of 160 mg of **1** in 8 ml of MeOH with excess CH_2N_2 in Et_2O for 16 hr at 4°, evaporation at red. pres. and recrystallization from $CHCl_3$–EtOH afforded 53 mg of the pyrazoline, mp 135–137° (dec.); MS *m/z* (rel. int.): 278 $[M]^+$, 260 (65.1), 135 (91.1), 107 (93.3), 91 (79.9), 79 (73.9), 41 (100).

Ambrosic acid (**2a**), mp 211° (dec.), lit. 220° [6], 211–213° [9]; IR ν_{max} cm^{-1}: 3164, 3121, 1726, 1698, 1620 and 880; MS *m/z* (rel. int.): 264 $[M]^+$ (20.1), 234 (100), 138 (60.2), 78 (77.4); ^{1}H NMR spectrum in Table 1. The ^{13}C NMR spectrum was consonant with that of the ethyl ester (**2b**) given in the literature [9]. A semisynthetic sample, mp 211–214°, was prepared from **1** by the HCl–Me_2CO method described in ref. [6] and was identical with the natural product.

Cumanin (**3**), mp 122–124° ($CHCl_3$–EtOH), lit. erratic [9]; IR ν_{max} cm^{-1}: 3448, 1713 and 1675; MS *m/z* (rel. int.): 266 $[M]^+$ (0.6), 248 (18.1), 230 (35.2), 215 (14.2), 107 (100). Chemical shifts and coupling constants in the 270 MHz ^{1}H NMR spectrum (Table 1) compared with the 60 MHz data given in ref. [10]; ^{13}C NMR spectrum in Table 2.

Psilostachyin C (**4**), mp 210–212° (dec.) and psilostachyin (**6**), mp 209–211° (dec.), were identified by comparison of R_fs, ^{1}H NMR and ^{13}C NMR spectra with those of authentic samples. Psilostachyin B (**7**) had mp 120–122°, lit. mp 123° [11]; IR ν_{max} cm^{-1}: 1764, 1734, 1659 and 1641; MS *m/z* 262 $[M]^+$, 247, 43, $[\alpha]_{25}^{\lambda}$ -9.30^{589}, -9.47^{578}, -9.05^{546}, $+2.30^{436}$, 42.52^{365} (*c* 1.17; $CHCl_3$). Chemical shifts and coupling constants in the 270 MHz ^{1}H NMR spectrum (Table 1) compared with data reported at lower field [15]; the ^{13}C NMR spectrum (Table 2) was essentially identical with that reported in ref. [5] except for the frequency of C-5 (δ86.7 instead of a reported 80.07).

Altamisic acid (**8a**), mp 141–142° (dec); IR ν_{max}^{KBr} cm^{-1}: 3200–2500 br, 1762, 1717 and 1650. Calc. for $C_{15}H_{20}O_5$: M_r 280.1310. Found: M_r (MS), 280.1294. Other significant peaks in the high resolution MS were at *m/z* (composition, %): 262 ($C_{15}H_{18}O_4$, 52.4) and 221 ($C_{13}H_{17}O_3$, 20.6); low resolution MS *m/z*: 280 $[M]^+$, 262, 251, 247, 234, 221, 108, 43 (100); $[\alpha]_D^{25}$ $+69.71^{589}$, $+72.58^{578}$, $+84.22^{545}$, $+158.78^{436}$; $+223.47^{365}$ (*c* 0.55, $CHCl_3$); ^{1}H NMR and ^{13}C NMR spectra in Tables 1 and 2. The methyl ester pyrazoline **9**, prepared as described for the pyrazoline of **1**, was a gum, MS *m/z* 308 $[M-N_2]^+$, 293, 290, 279, 235, 43 (100); ^{1}H NMR (80 MHz): δ5.19 (*d*, *J* = 7.2 Hz, H-6), 4.72 (*m*, 4H, H-13a, b, H-16a, b), 3.67 (3H, OMe), 3.29 (*ddd*, *J* = 7.2, 6, 12 Hz, H-7), 1.74 (3H, H-14), 1.48 (3H, H-15); ^{13}C NMR spectrum in Table 2.

Altamisin (**8b**), mp 89–91° (petrol–Me_2CO), lit. mp 101–104° [4]; IR ν_{max} cm^{-1}: 3496, 1770, 1721 and 1666; MS *m/z*: 308 $[M]^+$, 279, 262, 245, 221, 123, 109 and 43 (100); ^{1}H NMR ($CDCl_3$, 80 MHz) 6.20 (*d*, *J* = 4 Hz) and 5.50 (*d*, *J* = 2.7 Hz, H-13a, b), 4.72 (*d*, *J* = 9.6 Hz, H-6), 4.12 (*q*, *J* = 7 Hz, 2p, OEt), 3.36 (*m*, H-7), 1.73 (3H, *m*, H-14), 1.21 (3H, H-15), 1.25 (*t*, *J* = 7 Hz, OEt); the ^{13}C NMR spectrum in Table 2 was essentially identical with that reported in ref. [5] except for the frequency of C-5 (δ77.9 instead of a reported 86.5). We are unable to account for this discrepancy.

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