Phytochemistry, Vol. 20, No. 7, pp. 1601–1604, 1981.
Printed in Great Britain.

0031-9422/81/071601-04 $02.00/0

DAMSINIC ACID AND AMBROSANOLIDES FROM VEGETATIVE *AMBROSIA HISPIDA**

WERNER HERZ, DOUGLAS GAGE and NARENDRA KUMAR

Department of Chemistry, The Florida State University, Tallahassee, FL 32306, U.S.A.

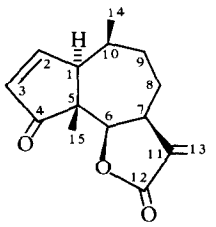
(*Received* 3 *October* 1980)

Key Word Index—*Ambrosia hispida*; Ambrosiinae; Compositae; sesquiterpene lactones; damsinic acid; ambrosin; damsin; 3-hydroxydamsin; kaurenes; hispidulin.

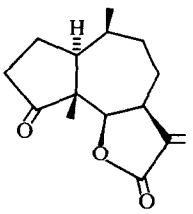
Abstract—*Ambrosia hispida* in the vegetative state furnished large quantities of damsinic acid and smaller amounts of ambrosin and damsin. Also isolated were anhydrocoronopilin, hispidulin and the new compounds 3-hydroxyambrosin damsinate and *ent*-12-oxokaura-9(11),16-dien-19-oic acid.

INTRODUCTION

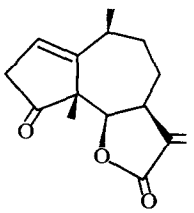
When collected at the flowering stage, *Ambrosia hispida* Pursh, a species of the Caribbean strand, was an excellent source of the sesquiterpene lactones ambrosin (**1**) and damsin (**2**) [1]. The flavone hispidulin was also found. Since ambrosin has interesting antitumor properties and since more of it was needed for metabolic studies, we secured additional plant material from the site of our earlier collection. However, the chloroform extract furnished much smaller amounts of **1** and **2** than previously and gave instead a very large quantity of damsinic acid (**4**) [2], possibly because the second collection was almost entirely in the vegetative state. In addition, we isolated anhydrocoronopilin (neoambrosin, **3**) [3,4], an unusual ester (**5**) of damsinic acid and 2-hydroxyambrosin and a new *ent*-kaurenoic acid (**6**) as well as a fairly large amount of the flavone hispidulin, which we had also found earlier [1].



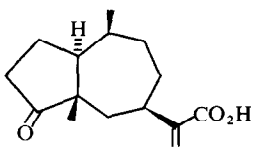
1



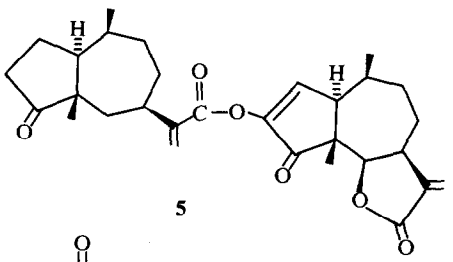
2



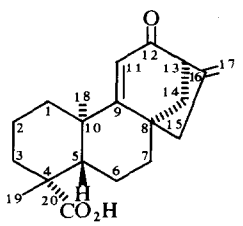
3



4



5



6

*Work supported in part by a grant from the U.S. Public Health Service (CA-13121) through the National Cancer Institute.

RESULTS AND DISCUSSION

The new ester **5**, $C_{30}H_{38}O_6$ (high resolution MS), mp 150–152°, was α,β-unsaturated (IR band at 1725 cm^{-1}) and also contained α,β-unsaturated γ-lactone, cyclopentanone and cyclopentenone functions (IR bands at 1770, 1750, 1705, 1645 and 1625 cm^{-1}). Comparison with the ^1H NMR spectra of **1**–**4** (Table 1) indicated that we were dealing with an ester of the unknown 3-hydroxyambrosin as the H-3 signal of **1** was missing and as the H-1 and H-2 signals of the ambrosin fragment had collapsed to a doublet of doublets and a doublet respectively. The sequence C-6, C-7, C-8, C-9, C-10, C-1, C-2 of the ambrosin half of **5** was established by spin decoupling in the usual way. The presence of a C–O bond at C-3 was apparent by comparing the previously unreported ^{13}C NMR spectra of **1** and its relatives with that of the ester (Table 2). As for the other half of the molecule, while overlap of the upfield signals in the ^1H NMR spectrum interfered with sequencing of the damsinic acid moiety, comparison of the ^{13}C NMR spectrum of **2** with that of **5** (Table 2) established unambiguously the nature of the esterifying acid. The absolute configuration is that shown in formula **4** since the absolute configurations of ambrosin and damsinic acid are known.

Acid **6** (*ent*-12-oxokaura-9(11),16-dien-19-oic acid), $C_{20}H_{26}O_3$ (MS), was very difficult to separate from damsinic acid and was isolated only in very small amount. IR and UV spectrometry indicated the presence of a carboxyl group (IR bands at 3120 and 1720 cm^{-1}), an α,β-unsaturated ketone (IR bands at 1650 and 1600 cm^{-1}), λ_{max} 250 and 322 nm (ε 15 000). The ^1H NMR spectrum which exhibited two methyl singlets at 1.18 and 1.30 ppm, two broadened singlets of a $=CH_2$ group at 5.25 and 4.25, and the slightly broadened singlet of system A suggests the presence of a tetracyclic diterpene. Also visible was a one-proton multiplet at 3.40 ppm, vicinally coupled (J s = 4.5, 2 Hz) to the protons of a methylene group one of which appeared as a doublet of doublets at 2.79 ppm (J s = 12, 4.5 Hz) and allylically coupled to the protons of $=CH_2$. The proton giving rise to this signal was tentatively identified as H-13 of kaurene **6** which, because of its position α to a ketone carbonyl on C-12, as in **6**, exhibited a greater than normal paramagnetic shift. This deduction was verified by the demonstration that H-13 was also long range coupled (J = 0.5 Hz) to the vinylic proton on C-11. After the conclusion of this work, a report appeared detailing the preparation of **6** by *t*-butyl chromate oxidation of grandiflorenic acid [5]. A direct comparison of the natural product with a synthetic sample established their identity.

Table 1. ^1H NMR spectral data of compounds **1**–**5***

	1	2	3	4†	5‡	
H-1	2.97 *ddd* (3, 2, 1.5)	2.0§	—	1.45 *m*§	**	3.00 *dd* (6, 2)
H-2	7.94 *dd* (6, 2)	2.0§	5.89 *dd* (2.5, 2)	1.45 *m*§	**	7.17 *d* (2)
H-3a	6.17 *dd* (6, 3)	2.4 *ddd* (21, 6, 2)	3.08 *dd* (21, 2)	2.13 *ddd* (18.5, 8.5, 2)	2.42 *ddd*§ (21, 9, 2.5)	—
H-3b	—	2.25 *m*‖	2.68 *dd* (21, 2.5)	1.81 *m*‖	2.22	—
H-6	4.66 *d* (10)	4.55 *d* (9)	4.35 *d* (10)	2.42 *dd* (14.5, 3) 1.51§	**	4.67 *d* (9)
H-7	3.42 *ddddd* (9, 5.5, 4), 3.5, 3.2)	3.32 *m*	3.33 *m*	2.75 *m*	2.77 *m*	3.46 *m*
H-8a	2.23 *dddd* (15, 8, 5.5, 2)	2.01§	2.0 *m*	1.82 *m*‖	**	2.25‖
H-8b	1.90 *dddd* (15, 9, 4, 2)	1.87	2.0 *m*	1.45§	**	1.92¶
H-9a	1.82 *m*		1.7 *m*	**	**	1.80¶
H-9b	1.69 *m*		1.7 *m*	**	**	1.70¶
H-10	2.42 *dsext* (1.7, 7)	2.22 *m*‖	2.81 *sext* (7)	1.71 *m*	**	2.42 *m*§
H-13a	6.31 *d* (3.5)	6.29 *d* (3.5)	6.10 *d* (3.5)	6.27 *br*	6.33 *br*	6.30 *d* (3.5)
H-13b	5.54 *d* (3.2)	5.55 *d* (3.2)	5.46 *d* (3.2)	5.35 *br*	5.73 *br*	5.51 *d* (3.2)
H-14	1.07 *d* (7)	1.11 *d* (7)	1.08 *d* (7)	0.81 *d* (7)	1.07 *d* (7)	1.09 *d* (7)
H-15	1.20	1.10	1.07	0.80	1.07	1.28

*Run at 270 MHz in $CDCl_3$, unless specified otherwise, with TMS as internal standard. Unmarked signals are singlets. Shifts are in ppm. Figures in parentheses are coupling constants in hertz.

†Run in C_6D_6.

‡First column refers to damsinate half, second column to 3-hydroxyambrosin half of molecule.

§, ‖, ¶Overlapping signals.

**Obscured signals.

Table 2. ^{13}C NMR spectra data of compounds **1–5**

Carbon	1	2	3	4	5	
1	48.35 *d*	46.22 *d*	149.17	46.55 *d*	46.23 *d*	42.83 *d*
2	163.62 *d*	24.02 *t*	124.43 *d*	22.75 *t*	22.51 *t*	143.84 *d*
3	131.19 *d*	36.05 *t*	39.89 *t*	35.92 *t*	35.81 *t*	145.90
4	210.70	218.21	213.87	220.75	220.47	201.84
5	56.56	55.01	58.46	51.30	50.95	55.22
6	80.89 *d*	81.79 *d*	79.85	38.41 *t*	38.13 *t*	79.87
7	44.97 *d*‡	44.62 *d*	43.49 *d*‡	36.83 *d*§	36.97 *d*§	44.40 *d*
8	25.32 *t*‡	25.89 *t*	23.95 *t*‡	34.34 *t*	34.07 *t*	24.68
9	30.37 *t*‡	33.63 *t*	30.19 *t*‡	31.21 *t*	30.84 *t*	29.90 *t*
10	34.12 *d*‡	34.52 *d*	38.78 *d*‡	35.15 *d*§	35.69 *d*§	33.78 *d*
11	139.03	140.11	139.07	147.03	146.77	138.54
12	170.67	170.02	169.91	171.73	163.59	170.11
13	119.84 *t*	120.36 *t*	119.63 *t*	124.12 *t*	124.70 *t*	119.45 *t*
14	17.57 *q*§	15.89 *q*	21.13 *q*¶	21.35 *q*‖	21.02 *q*‖	17.25 *q*§
15	17.44 *q*§	13.82 *q*	14.81 *q*¶	17.32 *q*¶	17.20 *q*‖	17.17 *q*§

*Run at 67.9 MHz in $CDCl_3$ with TMS as internal standard. Unmarked signals are singlets.
†First column refers to damsinate half, second column to 3-hydroxyambrosin half of molecule.
‡Assignment by single frequency off-resonance decoupling. Other assignments by analogy.
§Assignments may be interchanged.
¶Assignments deduced by SFORD and residual couplings in C_6D_6 solution.

The great difference in the relative amounts of ambrosin, damsin and damsinic acid from the two collections of *A. hispida* suggests the possibility that at least in this species damsinic acid serves as an intermediate in the biosynthesis of ambrosanolides.

EXPERIMENTAL

Extraction of Ambrosia hispida. Leaves and stems of *Ambrosia hispida* Pursh (the only flowering plant present served as the voucher), collected by J. Wassmer and D. W. Stevenson on 19 November *1979* on Lower Matecumbe Key, Monroe Co., Florida (Wassmer S.N. on deposit in herbarium of Florida State University), was extracted and worked up as described earlier [1]. The crude gum, wt 300 g from 9 kg of plant, was adsorbed on 380 g of silicic acid (Mallinckrodt 100 mesh) and chromatographed over 3 kg of silicic acid deposited with $CHCl_3$. Fractions (500 ml each) were collected as follows: 1–19 $CHCl_3$, 20–29 $CHCl_3$–MeOH (99:1), 30–38 $CHCl_3$–MeOH (97:3), 39–50 $CHCl_3$–MeOH (19:1). Fractions 1–19 contained waxy material and no lactones. Fractions 20–29 contained one major component which was purified by prep. TLC and recrystallized from $CHCl_3$–hexane, yield 0.5 g of damsin, mp 108–110°. Fractions 30–38 consisted mainly of ambrosin which required further purification by prep. TLC and/or recrystallization from EtOAc–hexane; yield of pure material 5.5 g, mp 145–147°.

Fractions 39–43 contained two substances which were separated by prep. TLC ($CHCl_3$–MeOH, 19:1, double development). The upper band contained **3** which was recrystallized from EtOAc–hexane, mp 125–126°, yield 250 mg identical with an authentic sample. The lower band contained **5** which was recrystallized from $CHCl_3$–hexane, yield 100 mg, mp 150–152°, IR (KBr) 1770, 1750, 1725, 1705, 1645, 1625, 1245, 1230 and 980 cm^{-1}; CD (MeOH) $[\theta]_{325}$ −14 820 (min), $[\theta]_{285}$ +49 490 (max), $[\theta]_{240}$ −51 200 (sh), $[\theta]_{212}$ −163 000 (last reading). (Calc. for $C_{30}H_{38}O_6$: MW, 494.2666. Found: MW, (MS) 494.2683, 2.1%). Other significant peaks in the high resolution MS were at *m/z* (composition, rel. int.): 479 ($C_{29}H_{35}O_6$, 3.1), 264 ($C_{15}H_{20}O_4$, 0.5), 248 ($C_{15}H_{20}O_3$, 4.9), 246 ($C_{15}H_{18}O_3$, 3.0), 235 ($C_{14}H_{19}O_3$, 27), 233 ($C_{15}H_{21}O_2$, 33.2), 233 ($C_{14}H_{17}O_3$, 100), 205 ($C_{13}H_{17}O_2$, 9.5), 204 ($C_{13}H_{16}O_2$, 17.5), 191 ($C_{12}H_{15}O_2$, 22.3), 190 ($C_{12}H_{14}O_2$, 15.4), 137 ($C_9H_{13}O$, 28.5), 125 ($C_7H_9O_2$, 23.2), 124 ($C_8H_{12}O$, 28.1), 123 ($C_8H_{11}O$, 79.5), 121 (C_9H_{13}, 36.8), 119 (C_9H_{11}, 38.7).

Fractions 44–48 on concn to smaller vol. deposited yellow crystals which were collected, recrystallized from aq. MeOH and identified as hispidulin, yield 2.5 g. Evapn of the filtrate gave a gum, wt 60 g, which consisted almost entirely of damsinic acid. TLC analysis indicated, in addition to **4** and traces of hispidulin, the presence of a component moving slightly faster than **4**. Repeated prep. TLC finally separated damsinic acid, mp 110–112° from EtOAc–hexane, from this unknown (**6**) which was crystallized from MeOH, yield 10 mg, mp 260–262° (dec.), IR ($CHCl_3$) 3120, 1720, 2650, 1600 900 cm^{-1}; UV λ_{max} 250 (ε 15 000), 322 nm; ^1H NMR signals (270 MHz, $CDCl_3$): δ 5.77 *br*, (*J* = 0.5 Hz, H-11), 5.25 *br*, 4.99 *br* (H-17), 3.40 *ddbr* (H-17), 3.40 *ddbr* (*J* 4.5, 2 Hz) 1.79 *dd* (*J* 12, 4.5 Hz, H-14a), 1.30 (C-4 Me), 1.18 (C-10 Me), 7.45 (—OH). Calc. for $C_{20}H_{26}O_3$: MW, 314.1881. Found: MW (MS), 314.1884, base peak. Other significant peaks in the MS were at *m/z* (composition, rel. int.): 299 ($C_{19}H_{23}O_3$, 25), 296 ($C_{20}H_{24}O_2$, 2.3), 283 ($C_{19}H_{23}O_2$, 6.6), 286 ($C_{19}H_{26}O_2$, 30.5), 272 ($C_{18}H_{24}O_2$, 8.3), 271 ($C_{18}H_{23}O_2$, 14.6), 269 ($C_{19}H_{25}O$, 4.4), 268 ($C_{19}H_{24}O$, 4.4), 259 ($C_{17}H_{23}O_2$, 4.6), 258 ($C_{17}H_{22}O_2$, 2), 257 ($C_{17}H_{21}O_2$, 3.7), 253 ($C_{18}H_{21}O$, 20.1), 225 ($C_{17}H_{21}$, 17.2), 197 ($C_{11}H_{17}O_3$, 11.6), 187 ($C_{10}H_{19}O_3$, 6.1), 169 ($C_{13}H_{13}$, 15.6), 161 ($C_{11}H_{13}O$, 18.9), 160 ($C_{11}H_{12}O$, 30.8), 159 ($C_{12}H_{15}$, 13.8), 159 ($C_{11}H_{11}O$, 9.9), 157 ($C_{12}H_{13}$, 16.2), 155 ($C_{12}H_{11}$, 14.4), 147 ($C_{10}H_{11}O$, 13.6), 146 ($C_{10}H_{10}O$, 18.7), 145 ($C_{10}H_9O$, 13.8), 143 ($C_{11}H_{11}$, 23.4), 141 ($C_{11}H_9$, 13.9), 133 ($C_{10}H_{13}$, 23.9), 132 ($C_{10}H_{12}$, 35.3), 131 ($C_{10}H_{11}$, 29.5). The substance was identical with a synthetic sample of **6** supplied by Professor MacMillan.

Acknowledgement—We wish to thank Professor Jake MacMillan for a sample of **6**.

REFERENCES

1. Herz, W. and Sumi, Y. (1964) *J. Org. Chem.* **29,** 3438.
2. Doskotch, R. W. and Hufford, C. W. (1970) *J. Org. Chem.* **35,** 486.
3. Herz, W. and Högenauer, G. (1961) *J. Org. Chem.* **26,** 5011.
4. Geissman, T. A. and Toribio, F. P. *Phytochemistry* **6,** 1563.
5. Lewis, N. J. and MacMillan, J. (1980) *J. Chem. Soc. Perkin Trans.* 1, 1270.

NOTE ADDED IN PROOF

Acid **6** also appears to be present in the extract of *Espeliotopsis guacharaca* (Diaz) Cuatr. as reported in an article which appeared after acceptance of this manuscript [Bohlmann, F., Suding, H., Cuatrecasas, J., Robinson, H. and King, R. M. (1980) *Phytochemistry* **19,** 2399] although it was obtained only in the form of the non-crystalline methyl ester. The latter was first prepared by oxidation of *ent*-11α-hydroxy-9(11),15(17)-kauradien-19-oic acid methyl ester [Bohlmann, F. and LeVan, N. (1978) *Phytochemistry* **17,** 1957].