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THE CHEMISTRY OF CONFERTIFLORIN AND THE MOLECULAR STRUCTURE OF CONFERTIFLORIN AND ALLODESACETYL-CONFERTIFLORIN, TWO MOLLUSCICIDAL SESQUITERPENE LACTONES

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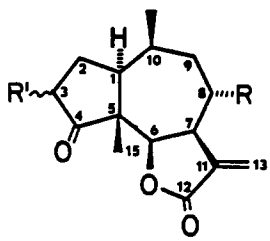
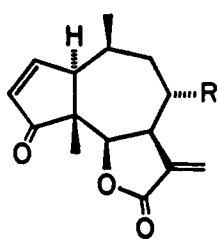
ABSTRACT.—The ambrosanolide confertiflorin (**2**) from *Ambrosia confertiflora* provided, upon treatment with toluenesulfonic acid, a mixture of desacetylconfertiflorin (**3**) and allodesacetylconfertiflorin (**5**). Treatment of confertiflorin with phenylselenyl chloride followed by H_2O_2 oxidation resulted in a mixture of 8α-acetoxyambrosin (**7**) and the dilactone 8α-acetoxy-2,3-dehydropsilostachyin C (**8**). The structures of the new compounds were inferred from nmr and mass spectral data. The molecular structures of the molluscicidal lactones confertiflorin (**2**) and allodesacetylconfertiflorin (**5**) were determined by single crystal X-ray diffraction.

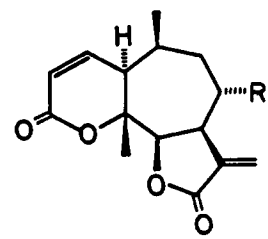
Snails of the genus *Biomphalaria* are the hosts in the life cycle of the blood fluke (genus *Schistosoma*, Schistosomatidae), which is responsible for human schistosomiasis (bilharzia), a disease affecting more than 200 million people in many tropical countries (1). It had been demonstrated earlier that the pseudoguaianolides damsin (**1**) and ambrosin (**6**) from *Ambrosia maritima* (Asteraceae) exhibited considerable molluscicidal activity (2), and, more recently, other sesquiterpene lactones were found to be active against snails of the genus *Biomphalaria* (3,4). In our continued search for molluscicidal plant natural products that could be used to control schistosomiasis-vectoring snails of the genus *Biomphalaria*, we have analyzed crude molluscicidal terpenoid extracts of



1 R=R′=H
2 R=OAc, R′=H
3 R=OH, R′=H
4 R=OAc, R′=-Se-Ph

5





6 R=H
7 R=OAc

8 R=OAc
9 R=H; 2,3-dihydro

Ambrosia confertiflora D C. for their active constituents. We describe in this paper the acid-mediated relactonization and oxidative modification of the ambrosanolide confertiflorin (**2**) (5). In addition, the molecular structures of the molluscicidal lactones confertiflorin (**2**) and allodesacetylconfertiflorin (**5**) are reported.

RESULTS AND DISCUSSION

Chromatographic procedures applied to the crude molluscicidal terpenoid extract of *A. confertiflora*[1] provided two lactonic constituents that were shown to be identical with confertiflorin (**2**) and desacetylconfertiflorin (**3**) by comparison (^{1}H nmr, ms, ir) with authentic compounds (5).

Chemical modifications of lactone **2** were carried out for the purpose of molluscicidal structure-activity relationship studies. Treatment of **2** with *p*-toluenesulfonic acid provided **3** and allodesacetylconfertiflorin (**5**) in a ratio of 11:9. Introduction of a 2,3-double bond was performed by modification of the procedure described by Sharpless and Michaelson (6) via the phenyl selenide derivative. Reaction of **2** with phenylselenyl chloride followed by H_2O_2 oxidation provided 8α-acetoxyambrosin (**7**), $C_{17}H_{20}O_5$, and 8α-acetoxy-2,3-dehydrospilostachyin C (**8**), $C_{17}H_{20}O_6$, in a ratio of 3:2. As in the ^{1}H-nmr spectrum of ambrosin (**6**) (7), lactone **7** exhibited typical absorptions (Table 1)

TABLE 1. ^{1}H-nmr Data of Lactones **7** and **8** (200 MHz in $CDCl_3$)

Proton	Compound	
	7	**8**
1	3.24 ddd (6.5,3.5,3)	3.01 ddd (5,2,2)
2	7.51 dd (6,3)	6.57 dd (10,2)
3	6.16 dd (6,3.5)	6.08 dd (10,3.5)
6	4.75 d (8.5)	4.93 d (10)
7	3.61 dddd (8.5,5.5,3.5,3)	3.68 dddd (10,10,4,3)
8	5.61 m (obs)	5.31 ddd (10,10,3)
9a	2.25 dd br (16,8)	2.09 m
9b	1.87 dd br (16,10)	2.01 m
10	2.47 m	2.41 m
13a	6.36 d (3.5)	6.31 d (4)
13b	5.59 d (3.0)	5.60 d (3)
14	1.08 d (8.0)	1.22 d (7.8)
15	1.20 s	1.33 s
CH_3-CO-	2.11 s	2.12 s

for protons on an α,β-unsaturated cyclopentenone moiety with two doublets of doublets at δ 7.51 (H-2: $J_{1,2}$=3.0 Hz; $J_{2,3}$=6.0 Hz) and 6.16 (H-3: $J_{1,3}$=3.5 Hz; $J_{2,3}$=6.0 Hz). The ^{13}C-nmr spectrum of **7** (Table 2) showed a very large downfield shift for C-2 and C-3 from the respective absorptions at δ 23.17 and 35.36 in **2** to δ 130.99 and 162.35 in **7**. The structure of the dilactone **8** was established by ^{1}H-nmr correlation with the known dilactone psilostachyin C (**9**) (8). Two doublets of doublets at δ 6.57 ($J_{1,2}$=2.0 Hz; $J_{2,3}$=10.0 Hz) and 6.08 ppm ($J_{1,3}$=2.5 Hz, $J_{2,3}$=10.0 Hz) were assigned to C-2 and C-3 of the unsaturated δ-lactone **8**, respectively. Further evidence for the attachment of a lactonic oxygen to C-5 in **8** was provided by ^{13}C-nmr chemical shift data for C-5 which appeared at 55.77 in enone **7** and at 87.51 in lactone **8** (Table 2).

[1]The molluscicidal activity was tested on *Biomphalaria glabrata* snails in the Lausanne laboratory according to the World Health Organization's recommendations. The following activities (LC_{100} in 24 h) were observed: crude syrups (100 ppm), lactones **2** and **8** (50 ppm), and lactone **5** (25 ppm).

TABLE 2. ^{13}C-nmr Data of Confertiflorin (**2**) and Derivatives (50 MHz in $CDCl_3$)[a]

Carbon	Compound				
	2	**3**	**5**	**7**	**8**
1	45.10 d	45.94 d	46.35 d	48.71 d	45.21 d
2	23.17 t	23.53 t	24.48 t	130.99 d	120.32 d
3	35.36 t	35.66 t	34.84 t	162.35 d	148.15 d
4	217.52 s	218.22 s	213.49 s	209.77 s	161.41 s
5	54.10 s	54.60 s	57.00 s	55.77 s	87.51 s
6	68.40 d	66.09 d	67.48 d	69.40 d	82.69 d
7	48.14 d	51.57 d	51.75 d	47.05 d	43.99 d
8	79.50 d	80.45 d	74.64 d	78.97 d	69.50 d
9	39.68 t	43.81 t	42.12 t	33.35 t	38.48 t
10	30.77 d	31.32 d	32.38 d	29.94 d	31.83 d
11	135.87 s	136.87 s	136.42 s	135.39 s	135.61 s
12	169.29 s[b]	170.09 s	169.46 s	169.58 s[b]	168.73 s[b]
13	122.76 t	123.86 t	121.65 t	121.23 t	122.35 t
14	13.31 q	13.66 q	15.55 q	16.63 q	14.33 q
15	15.41 q	15.79 q	17.35 q	17.24 q	17.35 q
16	169.03 s[b]	—	—	169.21 s[b]	169.79 s[b]
17	20.73 q	—	—	21.00 q	21.02 q

[a]Chemical shifts of carbon resonances were obtained under broad band decoupling conditions; multiplicities were derived from off-resonance decoupled spectra (s=singlet, d=doublet, t=triplet, q=quartet).

[b]Assignments are interchangeable.

CRYSTAL STRUCTURE ANALYSIS OF CONFERTIFLORIN (**2**).—The molecular structure of confertiflorin is illustrated in Figure 1, and its atomic coordinates are listed in Table 3. The conformation of the seven-membered ring is quite typical of pseudoguaianolides of the ambrosanolide class. It may be described as a half-chair with pseudo-twofold axis passing through C10 and bisecting the C6-C7 bond. This same conformation is found, for instance, in stramonin B (9), which exhibits a maximum difference in these seven torsion angles of 7.1°. Both five-membered rings of confertiflorin have half-chair conformations. The pseudo-twofold axis of the lactone passes through C12 and that of the cyclopentanone ring through C4.

Bond distances, which have individual standard deviations of 0.003-0.005 Å, and angles, with uncertainties 0.2-0.3°, are normal. Average distances for various bond

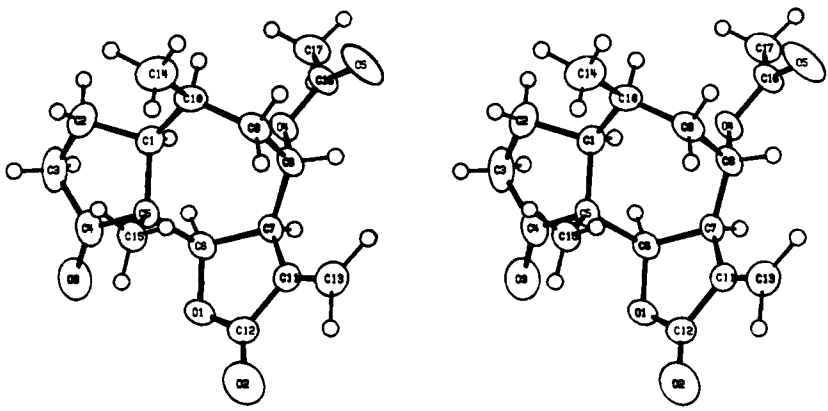


FIGURE 1. The Molecular Structure of Confertiflorin

TABLE 3. Coordinates for Nonhydrogen Atoms, Confertiflorin (**2**)

Atom	x	y	z	Atom	x	y	z
O1	0.1385(2)[a]	0.5586	0.3578(2)	C7	0.2783(2)	0.4447(3)	0.1916(3)
O2	−0.0504(2)	0.6219(3)	0.2161(3)	C8	0.3519(2)	0.3109(4)	0.1275(2)
O3	0.1933(4)	0.4557(4)	0.6530(3)	C9	0.2948(3)	0.1507(4)	0.1518(3)
O4	0.4965(2)	0.3204(3)	0.1836(2)	C10	0.3367(3)	0.0734(4)	0.2947(3)
O5	0.5608(2)	0.2182(4)	−0.0176(2)	C11	0.1345(3)	0.4764(4)	0.1229(3)
C1	0.3399(2)	0.1819(4)	0.4264(3)	C12	0.0603(3)	0.5591(4)	0.2306(3)
C2	0.3460(3)	0.0987(4)	0.5714(3)	C13	0.0769(3)	0.4452(5)	−0.0061(3)
C3	0.3366(3)	0.2275(5)	0.6787(3)	C14	0.2528(4)	−0.0750(4)	0.3064(5)
C4	0.2449(3)	0.3464(4)	0.5990(3)	C15	0.0771(3)	0.2453(4)	0.4075(3)
C5	0.2249(2)	0.3056(3)	0.4376(3)	C16	0.5907(3)	0.2699(4)	0.0986(3)
C6	0.2552(2)	0.4527(3)	0.3545(3)	C17	0.7346(3)	0.2848(5)	0.1675(4)

[a]Estimated standard deviations in the least significant digits are shown in parentheses.

types are 1.530 Å for $C(sp^3)$-$C(sp^3)$, 1.515 Å for $C(sp^3)$-$C(sp^2)$, 1.312 Å for C=C, and 1.196 Å for C=O. No unusually short intermolecular contacts exist.

CRYSTAL STRUCTURE ANALYSIS OF ALLODESACETYLCONFERTIFLORIN (**5**).—The molecular structure of **5** is illustrated in Figure 2, and atomic coordinates are given in Table 4. Both five-membered rings exist in conformations resembling the envelope. For the cyclopentanone ring, the resemblance is quite close, and C1 is at the flap. The lactone ring has C7 at the flap and slightly larger deviations from ideal C_s local symmetry. The conformation of the seven-membered ring is similar to the chair conformations found in guaianolides such as berlandin (10) and pumilin (11), which contain cycloheptene rings. The orientation of the pseudo-mirror differs, however, from those

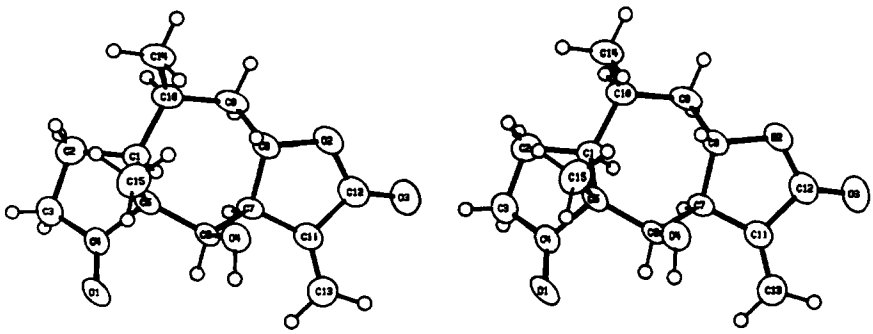


FIGURE 2. The Molecular Structure of Allodesacetylconfertiflorin

TABLE 4. Coordinates for Nonhydrogen Atoms, Allodesacetylconfertiflorin (**5**)

Atom	x	y	z	Atom	x	y	z
O1	0.0152(6)[a]	1.2671[b]	0.4724(3)	C7	0.1261(5)	0.9890(6)	0.1983(3)
O2	0.3008(4)	0.8334(5)	0.0731(2)	C8	0.3264(6)	0.9685(7)	0.1509(3)
O3	0.0910(5)	0.6300(6)	0.0355(3)	C9	0.3967(6)	1.1149(7)	0.0912(3)
O4	0.2328(4)	0.8971(4)	0.3774(2)	C10	0.4574(6)	1.2595(7)	0.1651(3)
C1	0.2999(6)	1.3037(6)	0.2465(3)	C11	0.0235(6)	0.8333(6)	0.1707(3)
C2	0.3308(7)	1.4711(7)	0.2973(4)	C12	0.1339(7)	0.7524(8)	0.0848(4)
C3	0.1758(7)	1.4825(7)	0.3797(4)	C13	−0.1375(7)	0.7681(8)	0.2072(4)
C4	0.1336(7)	1.3092(7)	0.4083(4)	C14	0.6663(6)	1.2398(8)	0.2134(4)
C5	0.2579(6)	1.1928(6)	0.3467(3)	C15	0.4357(8)	1.1621(8)	0.4250(4)
C6	0.1428(6)	1.0313(6)	0.3206(3)				

[a]Estimated standard deviations in the least significant digits are shown in parentheses.
[b]Fixed to define the origin.

structures, which exhibit a near-zero torsion angle about C1-C10, the position of the double bond. In allodesacetylconfertiflorin (**5**), which has a saturated seven-membered ring, there is a near-zero torsion angle (12.1°) about C5-C6, and the pseudo-mirror of the ideal C_s symmetry bisects this bond and passes through C9. There is a pronounced twist away from this ideal symmetry, leading to an asymmetry parameter (12) of 21.8°.

Bond distances have individual standard deviations 0.004-0.007 Å, bond angles 0.3-0.5°, and are normal. C(sp^3)-C(sp^3) bonds average 1.534 Å, C(sp^3)-C(sp^2) 1.500 Å, C=C 1.327 Å, and C=O 1.207 Å. The only intermolecular interaction of note is a hydrogen bond involving hydroxyl group O4 and cyclopentanone carbonyl oxygen O1 of a neighboring molecule related by the 2_1 axis at 0,y, ½. The O - - - O distance is 2.793(4) Å, the H - - - O distance is 1.92 Å, and the O-H . . . O angle is approximately 156°. This hydrogen bond links molecules in spiral chains running along the direction of the *b* axis of the crystal.

EXPERIMENTAL

A. confertiflora was collected on 18 June 1982, on US Hwy 90 between Hondo and Uvalde, Texas. (Fischer No. 135, voucher deposited at the Louisiana State University Herbarium.) The air-dried plant material (1600 g) was extracted and worked up as previously described (13), providing 18.3 g of crude terpenoid syrup. Chromatography on a silica gel column with $CHCl_3/Me_2CO$ mixtures of increasing polarity gave 55 fractions of 125 ml each. From fractions 15-28, 8.3 g of **2**, mp 142-143° [lit. (5) 145°] were isolated and fractions 23-40 provided 1.2 g of crude **3**, mp 185-188° [lit. (5) 202-204°].

DESACETYLCONFERTIFLORIN (**3**) AND ALLODESACETYLCONFERTIFLORIN (**5**).—Confertiflorin (250 mg, 0.817 m moles) was dissolved in 25 ml of MeOH and catalytic amounts of *p*-toluenesulfonic acid were added; the reaction was run under removal of methyl acetate by distillation. After 2 h, the starting material was consumed (tlc). The solvent was evaporated and the residue dissolved in $CHCl_3$, treated with aqueous 10% $NaHCO_3$, and washed with H_2O. The organic layer was dried over Na_2SO_4 and the solvent removed in vacuo to give 225 mg of an oily residue, which was separated by preparative tlc to provide 110 mg of **3** and 95 mg of **5**.

Confertiflorin (**2**): cd (c, 1.96×10^{-4}, MeOH) $[\theta]_{302}=+7.82\times10^{-4}$, $[\theta]_{243}=+2.6\times10^{4}$, $[\theta]_{211}=8.86\times10^{4}$.

Allodesacetylconfertiflorin (**5**): cd (c, 8.2×10^{-5}, MeOH) $[\theta]_{295}=1.34\times10^{3}$, $[\theta]_{218}=+1.63\times10^{3}$.

OXIDATIVE CONVERSION OF CONFERTIFLORIN (**2**) TO **7** AND **8**.—Confertiflorin (200 mg; 0.653 m moles) was dissolved in 25 ml EtOAc and 149 mg (0.784 m moles) of Ph SeCl were added and reacted at room temperature for 1 h, the reaction being monitored by tlc. The change in color of the solution from dark orange to yellow indicated completion of the reaction. The solution was washed with H_2O, and 0.8 m moles of aqueous 30% H_2O_2 were added to the organic layer. The mixture was reacted for 1 h when the solution changed from yellow to nearly colorless. The reaction mixture was washed with aqueous 5% $NaHCO_3$ followed by H_2O. The solvent was removed in vacuo leaving 195 mg of an oil that was separated by preparative tlc using $CHCl_3$-Me_2CO (9:1) as eluent, giving 110 mg of **7** and 83 mg of **8**.

8α-acetoxyambrosin (**7**), $C_{17}H_{20}O_5$, gum; cd (c, 3.29×10^{-4}, MeOH) $[\theta]_{331}=-1.87\times10^{2}$, $[\theta]_{251}=1.66\times10^{2}$, $[\theta]_{218}=-4.18\times10^{2}$; ir ν max (film) 1765 (γ-lactone), 1735 (ester); ms 70 eV *m/z* (rel. int.) 304 (2.5, M^+), 262 (25.4, M^+-CH_2=C=O), 244 (14.5, M^+-CH_3COOH).

8α-acetoxy-2,3-dehydropsilostachyin C (**8**), $C_{17}H_{20}O_6$, gum; cd (c, 9.6×10^{-5}, MeOH) $[\theta]_{259}=9.66\times10^{2}$, $[\theta]_{299}=8.11\times10^{2}$, $[\theta]_{211}=-2.14\times10^{3}$; ir ν max (film), 1780 (γ-lactone), 1760 (δ-lactone), 1720 (ester); ms 70 eV *m/z* (rel. int.) 320 (0.3, M^+), 278 (0.5, M^+-C_2H_2O), 260 (5.9, M^+-CH_3COOH).

X-RAY DATA.[2]—Data collection for both compounds, **2** and **5**, was performed on an Enraf-Nonius CAD4 diffractometer equipped with MoKα radiation (λ=0.71073 Å) and a graphite monchromator, by ω-2θ scans designed to yield $I\simeq 50\sigma(I)$ for all significant reflections. Crystal data are given in Table 5. Data in one quadrant were measured and corrected for background, Lorentz, and polarization effects; crystal decay and absorption were negligible.

The structures were solved using MULTAN (14) and refined by full-matrix least squares based on F, using data for which $I>3\sigma(I)$, weights $w=\sigma^{-2}(Fo)$, and the scattering factors of Cromer and Waber (15),

[2]Atomic coordinates for these structures have been deposited with the Cambridge Crystallographic Data Centre and can be obtained, on request, from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

TABLE 5. Crystal Data of Confertiflorin (**2**) and Allodesacetylconfertiflorin (**5**)

	Compound	
	2	**5**
Formula	$C_{17}H_{22}O_5$	$C_{15}H_{20}O_4$
Formula Weight	306.4	264.3
Crystal System	monoclinic	monoclinic
Space Group	$P2_1$	$P2_1$
a (Å)	9.692(3)	6.834(3)
b (Å)	8.646(2)	8.216(3)
c (Å)	9.351(1)	12.273(5)
β (deg.)	94.97(2)	93.62(3)
Z	2	2
Density (gcm^{-3})	1.303	1.276
μ (cm^{-1})	1.03	0.86
θ limits (deg.)	1-25	1-25
Unique Data	1478	1306
Observed Data	1229	859
Variables	218	171
R	0.035	0.047
Rw	0.046	0.056
Residual Density ($eÅ^{-3}$)	0.24	0.16

with the Enraf-Nonius SDP programs (16). Nonhydrogen atoms were treated anisotropically; H atoms were located by ΔF maps and included as fixed contributions with $B=5.0$ $Å^2$ for **5** and refined B's for **2**. Final R factors and residual electron densities are given in Table 5.

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