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MENTHOFURANS FROM *CALAMINTHA ASHEI* AND THE ABSOLUTE CONFIGURATION OF DESACETYLCALAMINTHONE

FRANCISCO A. MACIAS,* FRANK R. FRONCZEK and NIKOLAUS H. FISCHER†

Department of Chemistry, Louisiana State University Baton Rouge, Louisiana 70803, U.S.A.

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Key Word Index—*Calamintha ashei*; Lamiaceae; monoterpenes; menthofurans; desacetylcalaminthone; molecular structure; absolute configuration.

Abstract—Water washes from the aerial parts of *Calamintha ashei* provided, besides the known monoterpenes, menthofuran, (+)-epievodone and (−)-calaminthone, the new (+)-desacetylcalaminthone. The molecular structure and absolute configuration of desacetylcalaminthone was determined by single crystal X-ray analysis of its *p*-bromobenzoate derivative. Chemical correlations and chiroptical methods permitted determination of the absolute configuration of (−)-calaminthone and (+)-epievodone.

INTRODUCTION

The well drained sandy soils of Florida and the southeastern coastal plain support two vegetation types, which are distinctly different in species, composition and physioganomy [1]. The scrub is dominated by sand pine with a dense understory of evergreen oaks but little or no herbaceous ground cover. In contrast, the sandhill is an open wood and with scattered longleaf or slash pines and a complete ground cover of grasses and herbs. The ecotone between scrub and sandhill is abrupt and often devoid of vegetation.

We are currently testing a hypothesis [1] that relates the effects of fire and allelopathy in the two plant communities: allelochemicals released from scrub species deter the invasion of fire-prone sandhill grasses into the scrub, whereas frequent surface fires in the sandhill preempt the invasion of fire-sensitive scrub species into the sandhill community.

Calamintha ashei is a common endemic member of the Florida scrub with documented inhibitory effects on the germination and growth of sandhill grasses [2]. In the course of our directed search for germination and growth inhibitors of sandhill grasses by shrub species of the Florida scrub community [1, 3], we have previously isolated from *C. ashei* menthofuran-type monoterpenes which exhibited strong germination inhibitory activities on blue stem (*Schizachyrium scoparium*), a native grass of the sandhill community [4]. We report here the isolation and structure determination of a new menthofuran, desacetylcalaminthone (**2**). X-Ray diffraction analysis of its *p*-bromobenzoate derivative (**4**) established the molecular structure and the absolute configuration of desacetylcalaminthone. Acetylation of **2** provided calaminthone (**3**) [4] which also established the absolute configuration of **3**.

RESULTS AND DISCUSSION

Chemical studies

Fresh aerial parts of *C. ashei* were soaked in water at ambient temperature for 24 hr. The combined water extracts were exhaustively re-extracted with dichloromethane to provide a crude extract which was subjected to column chromatography over silica gel. Nonpolar fractions gave after further separations by preparative TLC (in order of elution), (+)-epievodone (**1**), menthofuran (**5**), calaminthone (**3**) [4] and a new monoterpene, desacetylcalaminthone (**2**) as well as the triterpene ursolic acid [4]. The structural identities of **1**, **3**, **5** and ursolic acid were confirmed by comparison of their physical data (mp, ^{1}H NMR, MS) with those of known standards.

Desacetylcalaminthone (**2**), $C_{10}H_{12}O_3$, is a crystalline compound (mp 102–103°) with strong IR absorptions at 3405 and 1684 cm^{-1}, indicating the presence of hydroxyl(s) and a conjugated carbonyl group, respectively. Comparison of the ^{1}H NMR and ^{13}C NMR spectra of **2** with the data for calaminthone (**3**) [4] (Tables 1 and 2) showed significant differences only for the signals associated with the hydroxyl group in **2** and the acetoxy moiety in **3**. Furthermore, the appearance of a diagnostic MS peak at m/z 162 in **2** $[M-H_2O]^+$ and **3** $[M-AcOH]^+$ assigned to cation **A** suggested that compound **2** represents the desacetyl derivative of **3**. Acetylation of terpene **2** with acetic anhydride in pyridine provided an acetate which was identical with natural calaminthone (**3**) [4]. However, comparison of the C-4 carbonyl absorptions in the ^{13}C NMR spectra of **1** (δ195.22s) and **2** (δ194.74s) with **3** (δ188.68s) (Table 2) revealed an unexpectedly large shielding effect of the acetoxy group in **3** upon the C-4 carbonyl carbon. This indicated that the acetoxy group in **3** must be in close proximity to the C-4 moiety suggesting that the acyloxy group is attached to C-5 instead of C-7 as was originally proposed on the basis of 200 MHz NMR data [5]. In order to remove structural ambiguities, a single crystal X-ray diffraction was performed on the *p*-bromobenzoate

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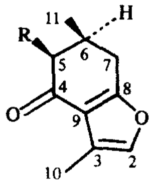
*Permanent address: Departamento de Quimica Organica y Biologia Molecular, Facultad de Ciencias, Universidad de Cadiz, Apdo. 40, 11080 Puerto Real, Cadiz, Spain.

†Author to whom correspondence should be addressed.

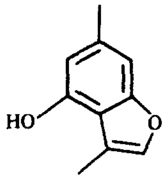
Table 1. ^{1}H NMR data of compounds **1**–**4** (400.13 MHz, CDCl$_3$, TMS as int. standard)

H	**1**	**2**	**3**	**4**
2	7.06 *br d*	7.13 *br d*	7.11 *br d*	7.14 *br s*
5α	2.48 *dd*	4.37 *dd*	5.55 *d*	5.78 *d*
5β	2.21 *dd*	—	—	—
6α	2.40 *ddddd*	2.81 *dddd*	2.70 *dddd*	2.86 *dddd*
7α	2.95 *dd*	3.16 *dd*	3.18 *dd*	3.27 *dd*
7β	2.47 *dd*	2.77 *dd*	2.77 *dd*	2.85 *dd*
10	2.17 *d*	2.19 *d*	2.17 *d*	2.17 *s*
11	1.15 *d*	0.94 *d*	1.08 *d*	1.19 *d*
OH	—	3.78 *d*	—	—
AC	—	—	2.20 *s*	—
14, 18	—	—	—	7.97 *d*
14, 17	—	—	—	7.60 *d*

J (Hz): compound **1**: 2,10 = 1.3; 5α,5β = 16.6; 5α,6α = 9.3; 5β,6α = 10.5; 6α,7α = 4.6; 6α,7β = 1.1; 6α,11 = 6.2; 7α,7β = 16.0. Compound **2**: 2,10 = 0.8; 5α,6α = 4.6; 5α,OH = 2.0; 6α,7α = 5.5; 6α,7β = 1.9; 6α,11 = 7.1; 7α,7β = 17.6. Compound **3**: 2,10 = 1.2; 5α,6α = 4.3; 6α,7β = 5.4; 6α,7β = 3.1; 6α,11 = 6.9; 7α,7β = 17.4. Compound **2a**: 5α,6α = 4.3; 6α,7α = 5.8; 6α,7β = 2.8; 6α,11 = 6.7; 7α,7β = 17.9; 14,15 = 17,18 = 10.6.



1 R = H
2 R = OH
3 R = OAc
4 *p* – Br – Ph –COO –
5 R = H; 4-desoxy.



A, *m/z* = 162

Table 2. ^{13}C NMR data of compounds **1**–**3** (100.62 MHz, CDCl$_3$, TMS as int. standard)*

C	**1**	**2**	**3**
2	139.07 *d*	140.35 *d*	140.21 *d*
3	118.95 *s*†	118.89 *s*†	119.31 *s*†
4	195.22 *s*	194.74 *s*	188.68 *s*
5	46.75 *t*	75.91 *d*	76.67 *d*
6	30.84 *d*	35.40 *d*	33.77 *d*
7	31.69 *t*	30.00 *t*	29.96 *t*
8	167.09 *s*	165.78 *s*	164.61 *s*
9	120.02 *s*†	117.95 *s*†	119.05 *s*†
10	8.95 *q*	8.71 *q*	8.70 *q*
11	21.02 *q*	11.97 *q*	13.85 *q*
1′			170.21 *s*
2′			20.76 *q*

*Carbon assignments were obtained by heteronuclear multipulse programs (DEPT) and ^{1}H–^{13}C chemical shift correlation.

†Assignments of quaternary carbons were made unambiguously by Pulsed Polarization Transfer via Long-Range ^{1}H–^{13}C couplings (INAPT) [7] optimized for $^{1r}J_{H-C} \simeq 8$ Hz.

derivative (**4**) which permitted determination of the absolute configuration of desacetylcalaminthone (**2**) and, by chemical correlation, of calaminthone (**3**). The X-ray results are discussed below.

Crystal structure analysis of desacetylcalaminthone, p-*bromobenzoate* (**4**)

The furan ring is planar, as is the aromatic ring of the *p*-bromobenzoate substituent. The cyclohexenone ring is nonplanar having an envelope conformation, with five atoms lying an average of 0.037 Å from a common plane, and C-6 lying 0.65 Å out of this plane. The C–O distances in the furan ring are asymmetric, with C-8-01 1.341 (4) Å and C-2-01 1.390 (5) Å. Likewise, the two double bonds of the furan ring have unequal lengths: C-2 = C-3 1.324 (6) Å; C-8 = C-9 1.356 (5) Å. The C–Br distance is 1.897 (3) Å.

The single crystal X-ray analysis of **4** established the molecular structure and absolute configuration of **2** and **3**, which required revision of the attachment of the acetoxy group in **3** from C-7β to C-5β. Although the $[\alpha]_D^{25}$ values for **2** (+37.5°) and **3** (−9.7°) had opposite signs, the CD spectra showed similar curves with a maximum at 284 and minima at 248 and 308 nm in **3** and maxima at 274 and 318 and minima at 242 and 293 nm in **2**. The optical rotation ($[\alpha]_D^{25}$ = +60°) of **1** was opposite to the rotation of synthetic (−)-evodone ($[\alpha]_D$ = −60°) [9]. Therefore, the methyl group at C-6 in **1** has to be β-oriented as in **2** and **3**. This is the first report of (+)-epievodone as a natural product.

EXPERIMENTAL

Plant material. Aerial parts of *Calamintha ashei* (Weatherby) Shinner were collected in June, 1987 in Polk County at Sun Ray, Florida (Donald R. Richardson; voucher deposited at the University of South Florida Herbarium at Tampa; sheet No. 137245).

Fresh *C. ashei* (1.0 kg) was soaked with H$_2$O (85 g/l) at room temp. for 24 hr, and the water re-extracted with CH$_2$Cl$_2$

(100 ml/l, × 10) to yield 5.0 g of crude extract which was subjected to CC over silica gel. Elutions with hexane and hexane–EtOAc mixtures of gradually increasing polarity provided 240 fractions of 30 ml each. Fractions 22–29 gave 70 mg of (+)-epievodone (**1**). Combined fractions 30–33 (65 mg) were further purified by prep. TLC on silica gel with hexane–EtOAc (9:1, × 3), giving epievodone (**1**) (6 mg), menthofuran (**5**) (3 mg) and calaminthone (**3**) (36 mg). Fractions 34–42 provided 80 mg of desacetylcalaminthone (**2**) and fractions 43–89 yielded after prep. TLC another 15 mg of **2** and ursolic acid (10 mg). Known compounds were identified by comparison of physical data (mp, ^{1}H NMR, MS) with those of known standards.

Epievodone (**1**). $C_{10}H_{12}O_2$, colourless crystals, mp 71–72° ($CHCl_3$); $[\alpha]_D^{25}+60°$ (MeOH; *c* 0.017); UV $\lambda_{max}^{H_2O}$ nm (log ε): 205 (3.65), 272 (3.51); CD (MeOH; *c* 0.017): $[\theta]_{250}-9.3\times10^4$, $[\theta]_{295}+3.5\times10^5$; MS (70 eV) *m/z* (rel. int.): 164 $[M]^+$ (41), 122 $[C_7H_6O_2]^+$ (100), 94 $[C_6H_6O]^+$ (77), 66 (24), 65 (27), 39 (27).

Desacetylcalaminthone (**2**). $C_{10}H_{12}O_3$, colourless crystals, mp 102–103° ($CHCl_3$); $[\alpha]_D^{25}+37.5°$ (MeOH; *c* 0.012); UV $\lambda_{max}^{H_2O}$ nm (log ε): 206 sh (3.79), 276 (3.47); CD (MeOH; *c* 0.012): $[\theta]_{242}-1.4\times10^5$, $[\theta]_{274}+3.9\times10^4$, $[\theta]_{293}-3.9\times10^4$, $[\theta]_{318}+5.8\times10^4$; IR ν_{max}^{heat} cm^{-1}: 3405 (OH), 1684 (conj. carbonyl); MS *m/z* (rel. int.) 180 $[M]^+$ (20), 162 $[M-H_2O]^+$ (10), 122 $[C_7H_6O_2]^+$ (100), 94 $[C_6H_6O]^+$ (63), 84 (35), 65 (24), 49 (50), 39 (29).

Preparation of calaminthone (**3**). Acetylation of **2** (3 mg) in pyridine–Ac_2O for 12 hr followed by usual work-up and prep. TLC gave 2 mg of **3** as a colourless gum [4]; $[\alpha]_D^{25}-9.7°$ (MeOH; *c* 0.031); UV $\lambda_{max}^{H_2O}$ nm (log ε): 206 sh (3.93), 280 (3.76); CD (MeOH; *c* 0.031: $[\theta]_{248}-3.6\times10^4$, $[\theta]_{284}+2.9\times10^4$, $[\theta]_{308}-4.3\times10^3$.

Desacetylcalaminthone, p-*bromobenzoate*, (**4**). Acylation of **2** (43 mg) in pyridine with 430 mg of *p*-bromobenzoyl chloride for 24 hr at ambient temp. gave after usual work-up and prep. TLC 10 mg of **4**, $C_{17}H_{15}O_4Br$, colourless crystals ($CHCl_3$); IR ν_{max}^{neat} cm^{-1}: 1726 (ester), 1696 (conj. carbonyl), 1267 (ester).

X-Ray data of desacetylcalaminthone, p-*bromobenzoate* (**4**). A crystal of dimensions 0.10 × 0.15 × 0.20 mm was used for data collection on an Enraf–Nonius CAD4 diffractometer equipped with CuKα radiation ($\lambda = 1.54184$ Å) and a graphite monochromator. Crystal data are: $C_{17}H_{15}O_4Br$, M_r 363.2, orthorhombic space group $P2_12_12_1$, $a = 10.5139$ (9), $b = 12.078(2)$, $c = 12.673$ (2) Å, $V = 1609.3$ Å^3, $Z = 4$, $d_c = 1.499$ g/cm^{-3}, $T = 23°$. Intensity data were measured by ω-2θ scans of variable rate designed to yield $I = 50\sigma(I)$ for all significant reflections. Octant *hkl* was collected within the limits $2° < \theta < 75°$, as well as octant *hk-l* with $h < 6$. Data reduction included corrections for background, Lorentz, polarization, decay (9.9%), and absorption. Absorption corrections ($\mu = 36.3$ cm^{-1}) were based on psi scans, with minimum relative transmission coefficient 82.78%. Of 2665 unique data, 2203 had $I > 3\sigma(I)$ and were used in the refinement.

The structure was solved by heavy atom methods and refined by full matrix least squares, treating nonhydrogen atoms anisotropically, using the Enraf-Nonius SDP [8]. Hydrogen atoms were located in difference maps and included as fixed contributions. Convergence was achieved with $R = 0.037$ and $R_w = 0.046$. The enantiomorphous structure was refined under identical conditions, yielding $R = 0.045$ and $R_w = 0.058$. Thus, the former

Table 3. Coordinates for desacetylcalaminthone *p*-bromobenzoate

Atom	*x*	*y*	*z*	B (Å^2)
Br	0.34266 (5)	0.06678 (4)	1.11631 (4)	6.46 (1)
01	0.4191 (4)	0.3643 (3)	0.2158 (2)	6.95 (8)
02	0.3370 (3)	0.3873 (3)	0.5702 (2)	6.44 (7)
03	0.4918 (3)	0.2197 (2)	0.6179 (2)	5.28 (6)
04	0.6195 (3)	0.3381 (3)	0.7001 (3)	7.55 (8)
C2	0.3259 (5)	0.4443 (4)	0.2312 (4)	7.3 (1)
C3	0.2999 (4)	0.4577 (3)	0.3326 (4)	6.1 (1)
C4	0.3998 (4)	0.3528 (3)	0.4979 (3)	4.67 (8)
C5	0.5116 (4)	0.2742 (3)	0.5179 (3)	4.64 (9)
C6	0.5284 (4)	0.1855 (4)	0.4331 (3)	4.84 (9)
C7	0.5490 (5)	0.2425 (4)	0.3264 (3)	5.8 (1)
C8	0.4508 (4)	0.3279 (3)	0.3122 (3)	5.02 (9)
C9	0.3824 (4)	0.3801 (3)	0.3880 (4)	4.89 (8)
C10	0.2074 (5)	0.5372 (4)	0.3802 (5)	8.2 (1)
C11	0.4178 (5)	0.1055 (4)	0.4298 (4)	5.4 (1)
C12	0.5439 (4)	0.2634 (3)	0.7029 (3)	4.84 (9)
C13	0.4973 (4)	0.2115 (3)	0.8010 (3)	4.48 (8)
C14	0.4077 (5)	0.1288 4)	0.8003 (3)	6.0 (1)
C15	0.3643 (5)	0.0843 (4)	0.8947 (3)	6.4 (1)
C16	0.4083 (4)	0.1234 (3)	0.9878 (3)	4.75 (9)
C17	0.4971 (5)	0.2045 (5)	0.9900 (4)	7.1 (1)
C18	0.5411 (5)	0.2479 (4)	0.8970 (4)	7.3 (1)

The equivalent isotropic thermal parameter, for atoms refined anisotropically, is defined by the equation: $4/3\,[a^2B_{11}+b^2B_{22}+c^2B_{33}+abB_{12}\mathrm{cos}y+acB_{13}\cos\beta+bcB_{23}\cos\alpha]$.

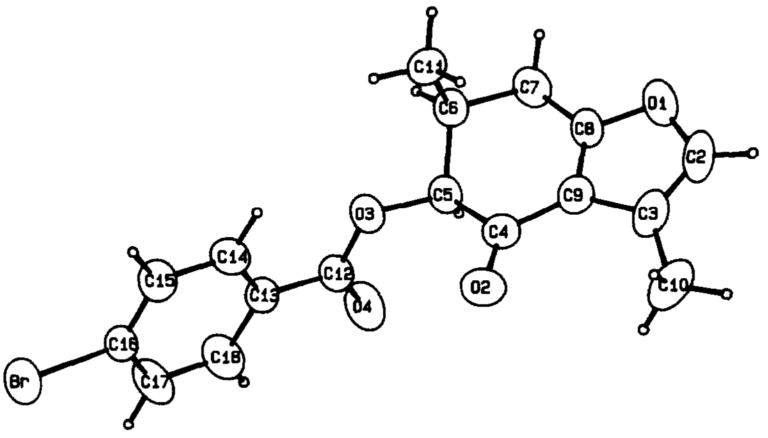


Fig. 1. The absolute configuration of desacetylcalaminthone, *p*-bromobenzoate (**4**).

was deemed correct; it is illustrated in Fig. 1, and its coordinates are tabulated in Table 3.

The crystal structure of the alcohol has also been investigated. It is orthorhombic, with cell dimensions $a = 5.467$ (3) Å, $b = 9.743$ (2) Å, $c = 17.403$ (2) Å, space group $P2_12_12_1$. Rapid intensity decay during data collection, presumed to result from sublimation, has prevented refinement beyond $R = 0.18$. The crystallographic data is deposited at the Cambridge Crystallographic Data Centre.

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