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NEW SESQUITERPENOIDS OF SWEET FLAG OIL (*ACORUS CALAMUS*)

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Key Word Index—*Acorus calamus*; Araceae; sweet flag oil; new guaiane sesquiterpenes; tropone; calamusenone.

Abstract—Two new sesquiterpenic ketones of the guaiane type have been isolated from Eastern European sweet flag oil (*Acorus calamus*) together with a new tropone, the structure of which reflects its sesquiterpenic origin.

INTRODUCTION

Sweet flag oil (*Acorus calamus*) represents a unique source of oxygenated sesquiterpenes of great structural variety and a large number of papers on its constituents have been published in recent years [1, 2]. The present communication describes the isolation and structure elucidation of two new sesquiterpenic ketones of the guaiane type, (**1**) and (**2**), from Eastern European sweet flag oil. Furthermore we report on a new tropone (**5**), the structure of which clearly reflects its sesquiterpenic origin. We suggest ketone **1** to be named calamusenone‡, as it represents a major component of the essential oil. As far as we know, none of these materials has previously been described in the literature or reported as naturally occurring.

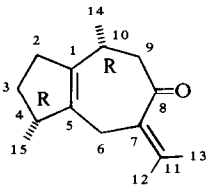
RESULTS AND DISCUSSION

Calamusenone (**1**) *and isomer* (**2**)

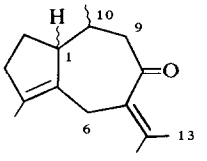
Compounds **1** and **2** were both isolated as colourless liquids from the high boiling cuts of sweet flag oil in 3.5 and 0.2% yield, respectively. Combination of MS, IR and NMR spectroscopy established their molecular formulae as $C_{15}H_{22}O$. IR and UV absorptions (**1**: 1675 cm^{-1}, 251 nm; **2**: 1675 cm^{-1}, 249 nm) were attributed to a conjugated unsaturated carbonyl unit. ^{13}C-NMR indicated the presence of four quaternary olefinic centres (**1**: δ 140.4, 138.8, 137.6, 134.5; **2**: 135.5, 135.5, 133.8, 132.6 ppm) and one carbonyl carbon (**1**: 204.6; **2**: 209.0 ppm), thus establishing the bicyclic skeletons for both compounds. PMR confirmed the absence of olefinic protons and revealed for **1** two secondary (δ 1.07, J = 7 Hz; 1.08 ppm, J = 7 Hz) and two olefinic methyl groups (1.85, 2.04 ppm), whereas the methyl absorptions of **2** appeared as three singlets (1.62, 1.76, 1.78 ppm) and one doublet (0.90 ppm, J = 7 Hz). Double resonance experiments at 100 MHz using a shift reagent ($Eu(FOD)_3$) at the same time permitted the assignment of the chemical shifts and J values for several protons. The secondary methyl groups of **1** were not attached to the same carbon as shown by these experiments. The PMR spectrum of **1** further indicated the presence of a doubly allylic methylene group appearing as a typical AB doublet at δ 2.95 and 3.17 ppm (J = 17 Hz, each). Similarly the doubly allylic protons of **2** exhibited an AB-pattern at 2.78 and 3.23 ppm (J = 16 Hz, each). The signals at 2.77 (2H, *d*, J = 6 Hz) and 2.30 ppm (2H, *m*) were assigned to the C-9 protons of **1** and **2**, respectively. In contrast to the strong effect of $Eu(FOD)_3$ on these protons, the doubly allylic C-6 methylene groups experienced only moderate downfield shifts induced by this reagent. As expected, the C-13 methyl protons of **1** and **2** also showed remarkable induced shifts towards lower field. For detailed NMR-data we refer to Table 2.

The combined spectroscopical information was in full accordance with the proposed structures as depicted by **1** and **2** for calamusenone and its double bond isomer. However, no evidence for configurational details could be obtained from these data. While the stereochemistry at C-1 and C-10 of **2** still remains unclear, unequivocal proof of the structure and absolute configuration of calamusenone (**1**) was achieved by X-ray crystallographic analysis of its *p*-bromophenylpyrazoline derivative (**3**) (M^+ *m/e* 387).

The colourless crystals of **3** have the space group $P\,2_12_12_1$ with a = 20.434, b = 8.548, c = 11.097 Å. Intensities were measured to $\theta \leqslant 20°$ with PDP8 controlled Hilger and Watts four-circle diffractometer using Zr filtered Mo-K_α radiation (λ = 0.71069 Å, μ = 2058 m^{-1}).



1



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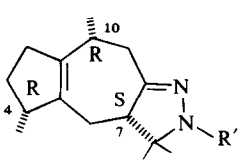


3 R′ = *p*-bromophenyl

‡IUPAC-nomenclature:3,8-dimethyl-5-(1-methylethylidene)-1,2,3,4,5,6,7,8-octahydroazulene-6-one.

Table 1. The final co-ordinates of the non-hydrogen atoms

Atoms	X	Y	Z
C-1	− 0.3686 (6)	− 0.1387 (18)	0.5693 (11)
C-2	− 0.3193 (6)	− 0.2003 (17)	0.6615 (11)
C-3	− 0.3610 (7)	− 0.2280 (19)	0.7740 (14)
C-4	− 0.4236 (7)	− 0.1277 (18)	0.7533 (11)
C-5	− 0.4250 (7)	− 0.1050 (16)	0.6211 (11)
C-6	− 0.4879 (7)	− 0.0392 (18)	0.5630 (11)
C-7	− 0.5000 (6)	− 0.1080 (16)	0.4417 (10)
C-8	− 0.4547 (6)	− 0.0561 (15)	0.3425 (11)
C-9	− 0.3855 (6)	− 0.0099 (16)	0.3632 (11)
C-10	− 0.3456 (5)	0·1272 (18)	0.4380 (10)
C-11	− 0.5681 (7)	− 0.0715 (16)	0.3833 (12)
C-12	− 0.5918 (8)	0.0928 (17)	0.4045 (15)
C-13	− 0.6173 (7)	− 0.1947 (19)	0.4251 (14)
C-14	− 0.2738 (7)	− 0.0686 (19)	0.4316 (13)
C-15	− 0.4214 (7)	0.0238 (18)	0.8225 (15)
N-16	− 0.5489 (5)	− 0.0931 (13)	0.2546 (9)
N-17	− 0.4814 (5)	− 0.0557 (14)	0.2393 (9)
Br-18	− 0.7136 (1)	− 0.1496 (2)	− 0.1967 (2)
C-19	− 0.6639 (4)	− 0.1123 (10)	− 0.0569 (6)
C-20	− 0.5958 (4)	− 0.1096 (10)	− 0.0661 (6)
C-21	− 0.5575 (4)	− 0.0969 (10)	0.0375 (6)
C-22	− 0.5873 (4)	− 0.0869 (10)	0.1503 (6)
C-23	− 0.6554 (4)	− 0.0896 (10)	0.1596 (6)
C-24	− 0·6937 (4)	− 0.1024 (10)	0.0560 (6)

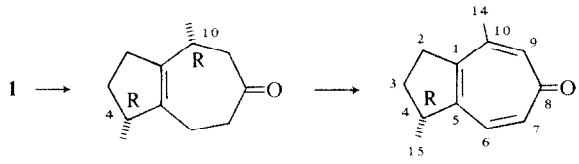
Independent data were obtained for 1078 planes of which 755 had $I \geqslant 2.5\ \sigma$ (I) and were used in the refinement. The structure was solved by MULTAN [3] and the remaining calculations were done mainly with SHELX [4]: both enantiomorphs were refined to convergence and that whose co-ordinates are in Table 1 had an R of 0.054 ($R' = 0.0533$) while the corresponding final values for the other enantiomorph were $R = 0.066$ and $R' = 0.066$. The absolute configuration given by the co-ordinates in Table 1 and shown in (**3**) may therefore be accepted as the correct one on the basis of the Hamilton R factor test [5].

Tropone (**5**)

Extraction of sweet flag oil with sulphuric acid afforded in small amounts* compound **5**† as a colourless, crystalline solid ($C_{12}H_{14}O$; M^+ *m/e* 174; mp 98–99°). IR and UV absorptions (1625, 1553, 1515 cm^{-1}; 235, 324 nm, log ε 4.468, 4.205) suggested the presence of a tropone moiety, a structural feature which has rarely been identified in natural products [6, 7]. Its PMR spectrum included the signals of one aromatic and one secondary methyl group (δ 2.27; 1.26 ppm, $J = 7$ Hz) and revealed three aromatic protons (7.02, *s*; 7.12, *d*, $J = 12$ Hz; 6.94 ppm, *dd*, $J = 12$ and 3 Hz). ^{13}C NMR indicated the presence of three tertiary (δ 140.3, 139.3, 133.9 ppm) and three quaternary olefinic centres (150.5, 148.1, 136.1 ppm). The δ value (186.5 ppm) for the carbonyl carbon was near the value reported for the same carbon in harringtonolide (δ 186.4 ppm) [7]. The spectroscopical evidence allowed us to establish the hydroazulene skeleton for **5** in an unambiguous manner. However, neither the positions of the two methyl groups nor the exact location of the carbonyl function could definitely be assigned by spectroscopical methods.

* *Ca* 7 ppm of the total essential oil.

† IUPAC-nomenclature: 3,8-dimethyl-1,2,3,6-tetrahydroazulene-6-one.



4 **5**

Final structure proof was achieved by chemical correlation of (+)-calamusenone (**1**) with the natural (−)-tropone. Treatment of **1** with EtOH–KOH (10%) afforded a ketone (**4**) (M^+ *m/e* 178), which was lacking the isopropylidene group (ν_{max} 1700 cm^{-1}). Bromination of **4** using pyrrolidone hydrotribromide (PHT) [8–10] followed by dehydrobromination using 1,5-diazabicyclo-(5.4.0)undec-5-ene (DBU) in DMSO [11] furnished a compound (**5**) identical in all respects (IR, UV, PMR, MS) to the tropone isolated from sweet flag oil. Moreover their identical modes of optical rotation established the (*R*)-configuration of the asymmetric C-4.

EXPERIMENTAL

GLC: Carbowax 20 M ($BaCO_3$-layer), 29 m × 0.27 mm WCOT glass column; He at 2–3 ml/min. PMR and ^{13}C NMR were recorded at 100 and 25 MHz, respectively. Eu(FOD)$_3$-d_{27} was employed for shift expts. The solvents used for spectral determinations were: $CDCl_3$–TMS (NMR); $CHCl_3$ (IR, $[\alpha]_D$); 95% EtOH (UV). TLC and PLC were carried out on pre-coated Si gel plates (Merck 60 F_{254}). Spots were detected by UV light and by spraying with conc H_2SO_4–H_2O (1:1). Merck Si gel 60 (70–230 mesh) was used for CC. No attempts were made to optimize yields of chemical transformations. All mps are uncorr.

Isolation of (+)-*calamusenone* (**1**) *and isomer* (**2**). Sweet flag oil of Eastern European origin (1 kg) was distilled under red. pres. and fractions boiling between 129 and 138°/0.03 mm were collected as a wide cut (350 g). CC on Si gel (6 kg) yielded 44 fractions by gradient elution using hexane–Et_2O mixtures. Part of combined fractions 26–30 (49 g), containing mainly **1** and (−)-acorenone [12, 13] was rechromatographed (7 g) on Si gel impregnated with $AgNO_3$ (15%) (2.1 kg). Elution with hexane–Et_2O (95:5) gave (**1**) (2.25 g) in pure state (GLC, isothermal 160°) as a colourless oil, bp 90°/0.01 mm. Fraction 33 (1.4 g), containing mainly **2**, was rechromatographed on $AgNO_3$–Si gel (600 g) using the same solvent system described above to afford **2** (300 mg) in a pure state (GLC, isothermal 160°) as a colourless oil, bp 90°/0.01 mm.

Compound **1**: $[\alpha]_D^{22}$ + 137° (*c* 0.83); UV λ_{max} nm (log ε): 211 (3.79), 251 (3.84); IR ν_{max} cm^{-1}: 2960, 2870, 1675 (=C—C=O), 1610, 1455, 1375, 1290; MS *m/e* (rel. int.): 218 [M^+] (100), 203(25), 189(12), 85(27), 176(38), 175(36), 161(50), 147(45), 133(46), 119(35), 105(50), 91(31), 83(20), 79(14), 77(15), 67(10), 58(8), 41(18).

Compound **2**: $[\alpha]_D^{22}$ − 178° (*c* 1.02); UV λ_{max} nm (log ε) 210(3.74), 249(3.685); IR ν_{max} cm^{-1}: 2960, 2870, 1675 (=C—C=O), 1615, 1455, 1375, 1300, 1260; MS *m/e* (rel. int.): 218 [M^+] (100), 203(45), 185(18), 175(45), 161(31), 147(55), 133(69), 119(26), 105(53), 91(39), 79(23), 77(24), 67(12), 55(13), 53(13), 41(22).

Pyrazoline derivative (**3**). To (+)-calamusenone (10 mg) in EtOH–HOAc (10:1) (1 ml) was added *p*-bromophenylhydrazine hydrochloride (40 mg) and the soln was stirred for 1.5 hr at 80°. Work up by adding dil. H_2SO_4, extraction into CH_2Cl_2 and evapn afforded a brown oil, which after purification by PLC (hexane–Me_2CO, 20:1) yielded **3** (10 mg) as colourless, prism-like crystals, mp 93–94° from hexane (M^+ *m/e* 387).

Isolation of tropone (**5**). A soln of sweet flag oil (1 kg) in Et_2O (1 l.) was extracted several times with 2N H_2SO_4. Neutralization of the combined H_2O phases in the cold with solid KOH, extraction into CH_2Cl_2 and evapn gave a brown oil (53 mg).

Table 2. NMR data of compounds 1 and 2*

Carbon	^{1}H NMR		^{13}C NMR	
	(1)	(2)	(1)	(2)
1		~2.82 (1H, *m*)	δ 138.789 *s*[b]	53.689 *d*
2	δ 1.38 (1H, *m*), 2.54 (1H, *m*)[a]	1.3–2.0 (2H, *m*)	34.266 *t*[c]	37.067 *t*[d]
3	2.04 (2H, *m*)[a]	2.20 (2H, *m*)	31.048 *t*[c]	23.420 *t*[d]
4	2.42 (1H, *m*)		45.052 *d*	135.451 *s*
5			137.617 *s*[b]	132.591 *s*
6	2.95 (1H, *d*, 17 Hz)	2.78 (1H, *d*, 16 Hz)	27.353 *t*	27.532 *t*
	3.17 (1H, *d*, 17 Hz)	3.23 (1H, *d*, 16 Hz)		
7			134.474 *s*	135.451 *s*
8			204.640 *s*	209.049 *s*
9	2.77 (2H, *d*, 6 Hz)	2.30 (2H, *m*)	48.926 *t*	47.555 *t*
10	~2.68 (1H, *m*)	~2.42 (1H, *m*)	33.372 *d*	31.095 *d*
11			140.402 *s*	133.787 *s*
12	1.85 (3H, *s*)	1.76 (3H, *s*)	22.546 *q*	20.623 *q*
13	2.04 (3H, *s*)	1.78 (3H, *s*)	23.003 *q*	21.989 *q*
14	1.08 (3H, *d*, 7 Hz)	0.90 (3H, *d*, 7 Hz)	19.844 *q*	16.626 *q*[e]
15	1.07 (3H, *d*, 7 Hz)	1.62 (3H, *s*)	19.733 *q*	13.948 *q*[e]

* Multiplicity in ^{13}C spectra obtained through off-resonance decoupling. [a–e] Assignments with the same superscript may be interchanged. Assignments were made through induced shift experiments with Eu(FOD)$_3$-d_{27}.

which after repeated separation and purification by PLC (CH_2Cl_2–MeOH, 20:1 and EtOAc) afforded **5** (7 mg), mp 98–99° from hexane–Et_2O. $[\alpha]_D^{22}$ – 12.8° (*c* 0.125); UV λ_{max} nm (log ε): 225(sh), 235(4.47), 324(4.205); IR ν_{max} cm^{-1}: 3000, 2965, 1625 (>C=O), 1595, 1553, 1515, 1445, 1390, 1255, 900, 845; MS *m/e* (rel. int.): 174 [M$^+$] (17), 146(9), 131(100), 115(13), 91(18), 77(8), 65(10), 51(8), 39(12); PMR: δ 7.12 (1H, *d*, *J* = 12 Hz), 7.02 (1H, *s*, C-9), 6.94 (1H, *dd*, *J* = 12 and 3 Hz), 3.20 (1H, *br q*, *J* = 7 Hz, C-4), 2.89 (2H, *m*, C-2), 2.27 (1H, *m*), 2.27 (3H, *s*, C-14), 1.58 (1H, *m*), 1.26 (3H, *d*, *J* = 7 Hz, C-15); ^{13}C NMR: δ 186.463 *s* (C-8), 150.461 *s*, 148.089 *s*, 146.063 *s*, 140.283 *d*, 139.337 *d*, 133.866 *d*, 44.281 *d* (C-4), 35.088 *t*, 30.511 *t*, 25.557 *q*, 20.154 *q*.

Conversion of calamusenone (**1**) *into ketone* (**4**). A soln of **1** (500 mg) in EtOH–KOH (10%) (20 ml) was heated to reflux for 12 hr. Work up by adding H_2O, extraction into CH_2Cl_2 and evapn gave a brown oil (480 mg), from which by PLC (3 plates, hexane–EtOAc, 4:1) ketone (**4**) (252 mg) was obtained as colourless needle-like crystals, mp 46–48° from hexane. $[\alpha]_D^{22}$ + 80.4° (*c* 1.08); UV λ_{max} nm (log ε): 215 (4.42); ν_{max} cm^{-1}: 2950, 1700 (>C=O), 1460; MS *m/e* (rel. int.): 178 [M$^+$] (69), 163(42), 150(27), 145(22), 135(31), 121(49), 107(72), 93(91), 91(59), 79(100), 77(51), 67(24), 65(23), 55(38), 53(31), 41(73); PMR: δ 1.8–2.9 (11H, *m*), 1.4 (1H, *m*), 1.10 (3H, *d*, *J* = 7 Hz), 1.02 (3H, *d*, *J* = 7 Hz). A small amount of the corresponding epimeric alcohols [colourless oil; 1:1-mixture by GLC, isothermal 175°; M$^+$ 180; ν_{max} cm^{-1}: 3600, 3440 (—OH)] was also recovered from the PLC plates, obviously as a result of a Meerwein–Ponndorf-type reduction of **4** during the process. In fact, ketone **4** was totally reduced to the corresponding alcohols by simply refluxing it in *iso*-PrOH–KOH (5%) for 6 hr.

Bromination–dehydrobromination of **4**. To a stirred soln of **4** (200 mg) in dry THF (5 ml) was added pyrrolidone hydrotribromide (PHT) (1.5 g) at 0°. Work-up after 10 min by introducing aq. $NaHCO_3$ and extraction into CH_2Cl_2 afforded after evapn a black tar (550 mg). Treatment of this material with DMSO–DBU (5:1) (6 ml) for 15 min at 60° yielded after work up (H_2O–Et_2O) and PLC (2 plates, EtOAc) tropone (**5**) (20 mg), mp 97–99° from hexane–Et_2O. $[\alpha]_D^{22}$ – 14.1° (*c* 0.375); MS, PMR, UV and IR spectra were identical with those of the tropone isolated from sweet flag oil.

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