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FIVE CHROMENES FROM *BLEPHARISPERMUM SUBSESSILE**

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Key Word Index—*Blepharispermum subsessile*; Compositae; chromenes; Oviposition deterrent activity; *Phthorimaea operculella*.

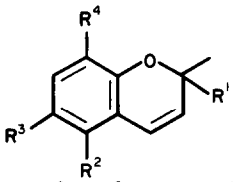
Abstract—Four new chromenes along with the known desmethoxyencecalin have been isolated from the acetone extract of *B. subsessile*. The new chromenes have been identified as 8-methoxy-2,2-dimethylchromene, desmethylisoencecalin, 5-hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene and (−)-artemesinol by spectral data. Desmethoxyencecalin exhibits oviposition deterrent activity against the potato tuber moth *Phthorimaea operculella*.

INTRODUCTION

The genus *Blepharispermum* Wight ex DC. belongs to the tribe Inuleae of the Compositae and includes 10 species [1]. No chemical work has been reported so far on this genus. During our screening programme of plant extracts for the isolation and identification of naturally occurring insect control agents, we have now examined *Blepharispermum subsessile* from the point of view of both insect control activity and chemistry.

RESULTS AND DISCUSSION

The acetone extract of the aerial parts of *B. subsessile* was subjected to screening for various activities against a number of insect pests. Notable activity observed was deterrence of oviposition by *Phthorimaea operculella* (potato tuber moth, PTM). Fractionation monitored by bioassay led to the isolation of squalene and five chromenes 1–5 and the oviposition deterrent activity was pinpointed to one of the chromenes identified as the known desmethoxyencecalin 3 [2–4]. The remaining four chromenes **1**, **2**, **4**, **5** are new natural products and their chracterisation by spectral data is discussed here.



1 R^1 = Me, R^2 = R^3 = H, R^4 = OMe

2 R^1 = Me, R^2 = OH, , R^3 = Ac, R^4 = H

3 R^1 = Me, R^2 = R^4 = H, R^3 = Ac

4 R^1 = CH_2OH, R^2 = OH, R^3 = Ac, R^4 = H

4a R^1 = CH_2OTs, R^2 = OTs, R^3 = Ac, R^4 = H

5 R^1 = $\beta-CH_2OH$, R^2 = R^4 = H, R^3 = Ac

5a R^1 = $\beta-CH_2OAc$, R^2 = R^4 = H, R^3 = Ac

8-*Methoxy*-2,2-*dimethylchromene* **1**

Compound **1**, obtained as a colourless liquid with a molecular formula $C_{12}H_{12}O_2$ (M^+ 190) exhibited in its ^1H NMR spectrum a singlet at δ1.4 (6H) assignable to two methyl groups, a singlet at δ3.77 (3H) due to a methoxy group, an AB quartet centered at δ5.58 and 6.37 attributable to the olefinic protons and a multiplet at 6.57–6.65 (3H) due to the aromatic protons. These spectral characteristics clearly reveal that **1** is a 2,2-dimethylchromene with a methoxy group on the aromatic ring. The ^1H NMR pattern due to aromatic protons further revealed that the methoxy group has to be either at C-5 or at C-8. Comparison of the ^1H NMR spectrum of **1** with that reported for 5-methoxy-2, 2-dimethylchromene [5] enabled us to place the methoxy group at C-8 position. Although **1** has been prepared synthetically [6–8] no spectral data are published and this is the first report of its isolation as a natural product. The ^{13}C NMR of **1** (Table 1) was in accordance with the structure assigned.

Desmethyl isoencecalin **2**

Obtained as plates, mp 103–104° (molecular formula $C_{13}H_{14}O_3$ M^+, 218), **2** showed positive $FeCl_3$ test indicating the presence of a phenolic group. Its IR spectrum displayed bands at 3000 (OH, confirmed by the IR spectrum after D_2O exchange), 1660 (C=O), 1645 (C=C), 1585 and 1485 cm^{-1} (aromatic ring). Its ^1H NMR spectrum showed a singlet at δ1.4 (6H) assignable to a *gem* dimethyl group, another singlet at δ2.48 (3H) attributed to an acetyl group, an AB quartet centered at δ5.577 and 6.604 (J = 10 Hz) (by spin decoupling studies) assignable to two olefinic protons, another AB quartet centered at δ6.33 and 7.518 (J = 8.8 Hz) (by spin decoupling studies) assignable to the ortho protons of the aromatic ring and a D_2O exchangeable singlet at δ14.35 due to phenolic group. The frequencies due to the hydroxy and carbonyl group in its IR spectrum and the chemical shift of the phenolic group in the ^1H NMR clearly

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indicated the intramolecular hydroen bonding between these two groups which was further confirmed by studying the IR spectra at different dilutions. All the above data suggested that 2 is also a 2,2-dimethylchromene in which the benzene ring is substituted with a hydroxy and an acetyl group *ortho* to each other. Literature survey revealed that 2 is identical with an intermediate in the synthesis of isoencecalin [9, 10] Mp, IR and ^{1}H NMR were in agreement with those of the reported for 2. The ^{13}C NMR spectrum was also in accordance with the structure assigned.

Desmethoxyencecalin 3 [2–4]

It was isolated as a viscous liquid (molecular formula $C_{13}H_{14}O_2$; M^+ 202) which was readily identified as desmethoxyencecalin by comparison of its spectral data and physical constants with those of reported [2] for desmethoxyencecalin. The ^{13}C NMR spectrum of 3 (Table 1) supported the structure. Compound 3 showed oviposition deterrent activity (see Experimental).

5-Hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene 4

Obtained as needles, mp 108°, analysing for $C_{13}H_{14}O_4$ (M^+, 234). It showed positive $FeCl_3$ test indicating that one of the oxygen atoms is present as a phenolic group. Its IR showed the presence of intramolecular hydrogen bonding as in 2 in addition to a free hydroxy group. Its ^{1}H NMR spectrum had a striking similarity with that of 2 except the *gem* dimethyl group 4 contained a CH_2OH group in place of one of the methyl groups at C-2 position, which was evident from the two singlets at δ1.4 (3H) and δ3.6 (2H) due to a methyl and a hydroxymethyl group respectively. The base peak in the mass spectrum of 4 *m/z* 203 (M − 31) and the downfield shift of the CH_2OTs in its tosylate (δ4.06) further supported the presence of CH_2OH group. The above data showed 4 to be 5-hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene.

(−)-*Artemesinol* 5

Obtained as needles. It had a mp 103° and molecular formula $C_{13}H_{14}O_3$ (M^+, 218). Its spectral data clearly revealed it to be identical with the known artemesinol [11] except for the rotation which was almost equal and opposite. The stereochemistry of artemesinol at C-2 has been established by its synthesis [11]. Hence 5 which is the optical antipode of artemesinol must be represented as 6-acetyl-2β-hydroxymethyl-2α-methylchromene. The ^{13}C NMR of 5 was in full accordance with this structure. It may be mentioned here that a chromene possessing the same mp and structure as 5 has been reported in literature [12]. Since its optical rotation has not been reported, it is not clear whether it is identical with 5 or its optical antipode.

EXPERIMENTAL

The plant material collected near Pune (Katraj ghat), India, was shade-dried and powdered. A voucher specimen has been deposited with NCL Herbarium. Mp's are uncorr. UV spectra were recorded in MeOH, ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$. TMS was used as the int. standard for the ^{1}H NMR spectra. IR spectra were recorded in $CHCl_3$ unless otherwise stated. Mass spectra were recorded at 70 eV.

Isolation of compounds. The powdered plant material (2 kg) was extracted with Me_2CO to yield the extract (100 g, 5%) of which 50 g was chromatographed over silicic acid using acetone: petrol (bp 60–80°) as the elution gradient. Five broad fractions A, B, C, D and E were collected of which C showed oviposition deterrent activity against PTM.

Squalene. Fraction A (1 g) was separated into two fractions by prep. TLC (Ag + SiO_2) and the less polar fraction was identified as squalene (50 mg, 0.005%) by comparison of its spectral data with those reported in the literature [13, 14].

Compound 1: Fraction B (10 g) on repeated chromatography afforded 1 (200 mg, 0.02%), bp 114–115°/2 mm. λ_{max}^{MeOH} (nm): 225, 262, 232 (ε 14800, 203, 176). ν max (liq. film) 3050, 2980, 1610, 1570, 1480, 1365, 1260, 1160, 1100, 965, 850, 815 cm^{-1}; ^{1}H NMR, Table 2; ^{13}C NMR Table 1; MS: *m/z* (rel int): 190 (M^+, 56), 175

Table 1. ^{13}C NMR spectral data of compounds 1–3 and 5 (in $CDCl_3$ at 25.05 MHz, TMS as internal standard, values in δ)

Carbon No.	1	2	3	5
C-2	75.68 (s)	77.858 (s)	77.598 (s)	80.782 (s)
C-3	131.67 (d)	131.864 (d)[b]	130.365 (d)[a]	130.695 (d)[a]
C-4	114.59 (d)[a]	114.122 (d)[c]	116.267 (d).	116.072 (d)
C-5	116.72 (d)[a]	159.94 (s)[a]	127.055 (d)[a]	127.315 (d)[a]
C-6	120.66 (d)[a]	118.023 (s)[d]	131.280 (s)	130.500 (s)
C-7	124.42 (d)	128.420 (d)[b]	128.420 (d)[a]	127.705 (d)[a]
C-8	151.17 (s)	116.072 (d)[c]	121.791 (d)	124.066 (d)[a]
C-9	158.67 (s)	159.84 (s)[a]	157.601 (s)	157.406 (s)
C-10	111.50 (s)	114.122 (s)[d]	120.751 (s)	120.556 (s)
C-11	27.57 (q)	28.465 (q)	28.400 (q)	26.191 (q)
C-12	27.57 (q)	28.465 (q)	28.400 (q)	68.824 (q)
Me	55.67 (q)	26.191 (q)	26.193 (q)	23.266 (q)
C=O	—	202.899 (s)	196.530 (s)	196.985 (s)

Signal multiplication in the single frequency off resonance decoupled (SFOD) spectrum.

[a–d] Interchangeable.

The signals are assigned tentatively by comparison with those reported for similar compounds.

(M−15, 100), 160 (M−30, 12), 144 (175−31, 7), 132 (42), 115 (11), 103 (10), 90 (9.5), 77 (14).

Compound **2**. In addition to **1**, fraction B on repeated chromatography yielded **2** (500 mg, 0.05%), mp 103° (lit 103–104° [9]). (Calc. for $C_{13}H_{14}O_3$; C, 71.54; H, 6.41. Found: C, 71.67; H, 6.5%). λ_{max}^{MeOH} (nm): 227, 235, 265 (ε 4250, 5270, 11920). $\nu_{max}^{CCl_4}$ 3000, 1660, 1645, 1586, 1488, 1460, 1390, 1270, 1110, 1075, 900, 875 cm^{-1}.

Deuteration of **2**. 10 mg of **2** was refluxed in 1 ml CCl_4 (dry) with D_2O (1 ml) for 30 min. The aq. layer was removed, fresh D_2O (1 ml) added and the refluxed for another 30 min. The aq. layer was removed and the solvent was evapd under vacuum to yield deuterated **2**: $\nu_{max}^{CCl_4}$ 3000 (OH), 2175 (OD) cm^{-1}. The intensity of the OH bond at 3000 cm^{-1} was decreased compared with the parent compound.

Compound **3**. Fraction C was repeatedly chromatographed to obtain **3** (600 mg, 0.06%), bp 140°/2mm. Its identity was established by comparison of its spectral data with those of reported for desmethoxyencecalin [2–4]. ^{13}C NMR, Table 1.

Compound **4**. Fraction D was rechromatographed to afford **4** (65 mg, 0.0065%), mp 108°, $[\alpha]_D$ + 5.656° ($CHCl_3$; *c* 0.35). (Calc. $C_{13}H_{14}O_4$; C, 66.62; H, 6.02. Found C, 66.78; H, 6.05%). λ_{max}^{MeOH} (nm) 238, 256, 315 (ε 4040, 14790, 6664). *v* max 3570, 3320, 3000, 2900, 1645, 1610, 1586, 1480, 1420, 1265, 1040 cm^{-1}. ^1H NMR Table 2. MS: *m/z* (rel. int.) 234 (M^+, 10), 218 (M−16, 5), 203 (M−31, 100), 189 (218−29, 11), 187 (218−31, 25), 185 (M−31−18, 47), 109 (56), 108 (57), 91 (47), 71 (40).

Tosylation of **4**. A solution of 20 mg of **4** in pyridine (0.5 ml) was cooled to 0° and to it was added *p*-toluenesulphonyl chloride (40 mg) and left as such overnight. The usual work-up furnished **4a** (10 mg). ^1H NMR Table 2.

Compound **5**. In addition to compound **4** fraction D on rechromatography yielded compound **5** (400 mg, 0.04%), mp 103° $[\alpha]_D$ − 4.62° ($CHCl_3$; *c* 1.6). (Calc. C, 70.88, H, 6.67; found: C, 70.54; H, 6.47%). λ_{max}^{MeOH} (nm) 228, 273, 315 (ε 3093, 5616, 2316). ν_{max} 3580, 3450, 2990, 1670, 1645, 1600, 1550, 1485, 1365, 1270, 1180, 1040 cm^{-1}. ^1H NMR Table 2, ^{13}C NMR Table 1. MS: *m/z* (rel. int.), 218 (M^+, 3.5), 203 (M−15, 3), 187 (M−31, 100), 175 (203−28, 34), 144 (187−43, 44), 105 (22), 99 (6).

Acetate of **5**. Compound **5** (50 mg) was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) at room temp. The usual work-up gave **5a** which was purified by prep. TLC, mp 48–50°, $[\alpha]_D$ − 62.76° ($CHCl_3$; *c* 0.62). ν_{max} 2990, 2920, 1730, 1680, 1620, 1575, 1500, 1360, 1266, 1230, 1100, 830 cm^{-1}. ^1H NMR Table 1.

Bioassay for assessing oviposition deterrence. Test tubes (20 × 2.5 cm) were covered with black muslin cloth which served as a preferred substrate by the test insect PTM. The covering black muslin cloth was impregnated with the test material, 5 mg/cm^2, using Me_2CO as solvent. 5–10 test tubes (replicates) were prepared as above with 2 controls in which the covered muslin cloth was left untreated. When Me_2CO was completely evapd from each test tube, equal numbers of 0–24 hr-old male and female PTM were introduced in each test tube. Food was provided in the form of cotton swabs in 20% honey, which was changed after every 48 hr. Number of eggs laid on the treated and untreated surfaces were counted after 48 hr and percentage of oviposition deterrence was calculated by using the formula: $[T - E/T] \times 100$ where T = total number of eggs laid both on treated and untreated substrate; and E = number of eggs laid on treated substrate. When 100% oviposition deterrent activity was observed till 48 hr, these observations were extended till oviposition occurred on the treated substrate. After every week, the old batch of insects was replaced by the same number of fresh 0–24 hr-old male and female insects.

By following the above method, desmethoxyencecalin **3** was found to exhibit 10 days' oviposition deterrent activity.

Table 2. ^1H NMR spectral data of **1**, **4**, **5** and their derivatives

Proton	1	4	4a	5	5a
H-3	5.58 *d* (10)	5.47 *d* (10)	5.56 *d* (10)	5.60 *d* (10)	5.56 *d* (10)
H-4	6.37 *d* (10)	6.81 *d* (10)	6.72 *d* (10)	6.42 *d* (10)	6.44 *d* (10)
H-5–H-7	6.57–6.65 *m*			7.60 *m*	7.64 *m*
		7.46 *d* (8.8)	7.79 *d* (8.8)	7.60 *m*	7.64 *m*
H-8		6.27 *d* (8.8)	6.5 *d* (8.8)	6.72 *d* (8)	6.75 *d* (8)
H-11	1.4	1.4	1.44	1.42	1.45
H-12	1.4	3.6	4.06	3.68	4.12 *q* (2.5, 11)
Me	3.77				
Me		2.48	2.54	2.55	2.5
OH		10.44			
OTs			7.35 *d* (8)		
			7.69 *d* (8)		

Figures in parentheses denote coupling constants values in δ.

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