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A NEW MELAMPOLIDE FROM *SIGESBECKIA ORIENTALIS**

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Key Word Index—*Sigesbeckia orientalis*; Compositae; Helianthinae; Melampodiinae; melampolide; sesquiterpene lactone.

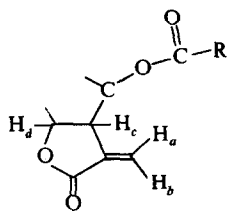
Abstract—Isolation and structure determination of orientalide, a new melampolide closely related to the acanthospermals, from *Sigesbeckia orientalis* is reported.

INTRODUCTION

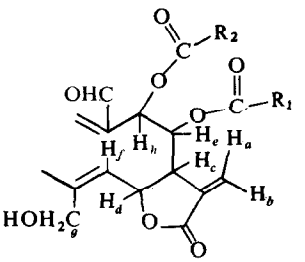
Sigesbeckia orientalis L., Sens. lat. [1] is widely used as a medicinal plant and contains the diterpene glycoside darutoside [2–6]. There is also a recent report [7] on a sesquiterpene lactone **1** of unknown stereochemistry. The name orientin selected by the Russian workers for their sesquiterpene lactone from *S. orientalis* is preempted by a *C*-glycosylflavonoid. We now describe isolation and structure determination of a new melampolide, **2a**, which we have named orientalide.

RESULTS AND DISCUSSION

Orientalide **2a**, $C_{21}H_{24}O_8$ $[\alpha]_D^{25}$ + 41.2° was an α,β-unsaturated aldehyde (IR band at 1690 cm^{-1}, ^1H NMR signal at 9.49 ppm) and an α,β-unsaturated lactone of the type shown in **A** as evidenced by the usual criteria (strong UV end absorption due to superposition of two chromophores) IR band at 1780 cm^{-1}, narrowly split doublets at 6.26 and 5.84 ppm in the ^1H NMR spectrum (Table 1).



A



B

The location of H_c as a multiplet at 2.65 ppm was established by double irradiation at the frequency of H_a and H_b. Irradiation at the frequency of H_c collapsed H_a and H_b into singlets and also converted a triplet at 5.34 ppm ($J_1 = J_2 = 10.5$ Hz) into a doublet and a narrowly split doublet of doublets at 6.74 ppm ($J_1 = 9$, $J_2 = 1.0$ Hz) into a doublet ($J = 9$ Hz). The relative shifts of H_d and H_e indicated that H_d represented the proton under the lactone ether oxygen, whereas H_e was attached to a carbon atom carrying one of the two ester functions whose presence was indicated by IR bands at 1730 and 1720 cm^{-1}.

Table 1. ^1H NMR spectra of orientalide **2a** and its acetate **2b**

Protons	**2b**	**2b**
H-1	6.81 *dd* (9, 8)	6.74 *dd* (9, 8)
H-2a	2.81 *m*	2.92 *m*
H-2b	2.69 *m*	2.70 *m*
H-3a, b	1.95 *m**	1.95 *m*
H-5	5.03 *d* (10.5)	5.18 *d* (10.5)
H-6	5.34 *t* (10.5)	5.18 *t* (10.5)
H-7	2.65 *m*	2.70 *m*
H-8	6.74 *dd* (1, 9)	6.70 *dd* (1, 9)
H-9	5.44 *dd* (9, 2)	5.36 *dd* (9, 2)
H-13a	5.84 *d* (3)	5.85 *d* (3)
H-13b	6.26 *d* (3)	6.28 *d* (3)
H-14	9.49 *d* (2)	9.48 *d* (2)
H-15†	4.51	4.87
H-3′a	5.60 *m*	5.60 *m*
H-3′b	6.06 *br*	6.04 *br*
H-4′‡	1.92 *br*	1.92 *br*
OAc	1.95	1.92, 2.12

Run in $CDCl_3$ at 270 MHz on a Bruker HX-270 instrument. Values are in ppm, *d*, doublet; *t*, triplet; *br*, broadened singlet. Unmarked signals are singlets. Values in parentheses are coupling constants in hertz. *Intensity three protons. †Intensity two protons centre of AB system, J_{AB} = 10.5 Hz. ‡Intensity three protons.

Expansion of **A** to partial structure **B** was made possible by further spin decoupling experiments. Irradiation at the frequency of H_d converted H_e into a broad singlet and a broadened doublet at 5.03 ppm (J = 10.5 Hz H_f) into a broadened singlet. Irradiation at the frequency of H_e sharpened H_c and also converted a doublet of doublets at 5.44 ppm (H_h $J_1 = 9$, $J_2 = 2$ Hz) into a doublet (J = 2 Hz). The smaller coupling of H_h could be traced to the aldehyde proton H_i.

The ^1H NMR spectrum further exhibited a one-proton doublet of doublets at 6.18 ppm ($J_1 = 9$, J_2 = 8 Hz) presumably the proton β to the aldehyde function. Irradiation at the frequency of H_i simplified two well

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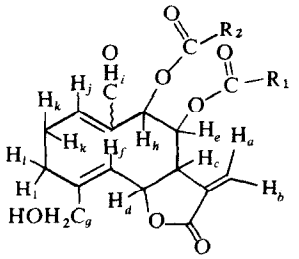
separated multiplets at 2.81 and 2.69 ppm which could be assigned to geminally coupled methylene protons (H_k). Decoupling experiments further showed that the H_k protons were adjacent to another methylene group whose signal appeared as a multiplet at 1.95 ppm.

The ^{1}H NMR spectrum of orientalide also revealed the presence of a vinyl methyl multiplet at 1.92 ppm, two vinyl proton multiplets at 5.60 and 6.06 ppm. Irradiation at 6.06 ppm simplified the multiplets at 5.60 and sharpened the broad singlet at 1.92, thus suggesting the presence of an OCOC(Me)=CH_2 group which would represent one of the two ester functions. This deduction was strengthened by the presence in the high resolution MS of **2a** peaks at *m/e* 69 (C_4H_5O) and at *m/e* 318 due to loss of $C_4H_6O_2$ from the molecular ion.

Two additional features of the ^{1}H NMR spectrum remain to be mentioned. One was a three proton singlet at 1.95 ppm ascribable to an acetyl group whose presence was indicated by an IR band at 1730 cm^{-1}. The second was a two-proton AB system centred at 4.5 ppm which underwent a paramagnetic shift to 4.87 on acetylation to **2b**.

Consideration of these results permitted extension of partial structure **B** to **C**, all of whose features are in agreement with the ^{13}C NMR spectrum (Table 2) and resemble those of acanthospermal B **2c** from *Acanthospermum hispidum* DC. except for the nature of one of the ester groups. That orientalide, like acanthospermal B, was a melampolide could be shown in the following manner. The chemical shift of H-14 (9.49 ppm) indicated



C

that the 1(10)-double bond was *cis*; in a *trans* aldehyde the signal would have appeared at 10 ppm or higher [8]. The ^{1}H NMR spectrum of the dialdehyde **3** obtained by MnO_2 oxidation of **2a** exhibited the new aldehyde proton at 10.15 ppm indicating that the 4,5-double bond was *trans*. Because of the coincidence of chemical shifts and coupling constants, the arguments previously made for the stereochemistry of acanthospermal B [9] also hold for orientalide and will therefore not be discussed in detail. Consequently, orientalide was represented by **2a** where the only remaining element of uncertainty was the distribution of ester groups at C-8 and C-9.

Table 2. ^{13}C NMR spectra of orientalide **2a**

Carbon	2a	Carbon	2a
C-1	158.89 *d*	C-12	169.99
C-2	27.57	C-13	122.45 *t*
C-3	32.39 *t*	C-14	193.86 *d*
C-4	140.68	C-15	60.58 *t*
C-5	128.42 *d*	C-1′	165.74
C-6	72.40 *d*	C-2′	135.20
C-7	50.94 *d*	C-3′	126.77 *t*
C-8	69.85 *d*	C-4′	18.30 *q*
C-9	68.85 *d*	C-1″	170.45
C-10	141.36	C-2″	20.67 *q*
C-11	133.60		

Run in $CDCl_3$ at 67.9 MHz on Bruker HX-270 instrument. Unmarked signals are singlets. Assignments based on analogy to acanthospermal A and B.

In order to decide the locus of the two ester side chains, we attempted to follow the procedure successfully used for acanthospermal A and B. However, in the case of orientalide, selective displacement of the ester function on C-9 with retention of the ester at C-8 was not possible because of the greater susceptibility of the methacrylate carbonyl toward nucleophilic attack. Thus hydrolysis of **2a** with K_2CO_3–MeOH or KOH–MeOH gave the same product $C_{17}H_{24}O_7$ by allylic displacement of the ester function on C-9, hydrolysis of the ester function on C-8 and addition of methanol to the methylene of the α,β-unsaturated lactone. It should be noted that whereas in our work on the acanthospermals [9] we considered only formulae analogous to **5a**, formulae analogous to **4a**, the result of an $SN^{2'}$ displacement of the acetate, cannot be excluded.

To decide between **4a** and **5a**, the hydrolysis product was oxidized first with MnO_2 to the corresponding dialdehyde, which was further oxidized with DMSO and acetic anhydride to a ketoaldehyde whose IR spectrum exhibited a much stronger band at 1685 cm^{-1} than its precursor. Because the dramatic increase in intensity of this band could arise from superposition of new α,β-unsaturated ketone absorption on two α,β-unsaturated aldehyde frequencies, we tentatively favour formulae **4a**, **6** and **7** for the hydrolysis and its transformation products although this has no bearing on the structure of orientalide itself. The small scale on which the transformations of **4a** were carried out prevented characterization of **7** by high resolution NMR spectrometry.

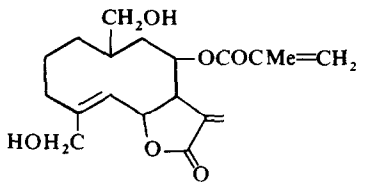
While the ease with which the 4-carbon ester side chain is hydrolysed argued in favour of its attachment to C-8, additional proof was desirable. An attempt was therefore made to reduce the methacrylate to an isobutyrate ester which is known to be resistant to hydrolysis under mild conditions [9, 10]. Hydrogenation of **2a** led to a mixture from which a substance whose properties corresponded to **8** was isolated as the major component. The loss of the acetate function by hydrogenolysis requires that it be allylic to the 1(10) double bond, hence attached to C-9 and definitely permitted placement of the methacrylate ester side chain at C-8 as in **2a**. Finally, the behaviour of orientalide toward $NaBH_4$ reduction to give **9a** was similar to that of the acanthospermals.

With the exception of enhydrin from *Enhydra fluctuans* Lour (Ecliptinae), all melampolides reported to date have been isolated from species belonging to subtribe Melampodiinae. The occurrence of orientalide in *S. orientalis* may have taxonomic implications by conceivably providing chemical evidence for the recent transfer of *Sigesbeckia* from Helianthinae to Melampodiinae [11].

EXPERIMENTAL

Mps are uncorr. For PLC, Si gel G was used.

Extraction of Sigesbeckia orientalis. Above ground parts (500 g) collected on 4 October, 1977 at Nitaipukhuri, Mariani, Assam, were first extracted with CH_2Cl_2 for 16 hr and then with



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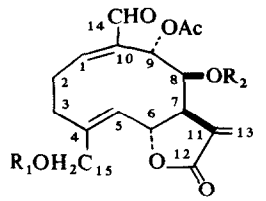
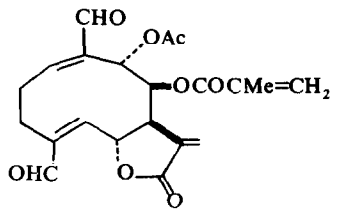


2a $R_1 = H$; $R_2 = R_2 = COCMe{=}CH_2$
2b $R_1 = Ac$; $R_2 = R_2 = COCMe{=}CH_2$
2c $R_1 = H$; $R_2 = R_2 = COCHMeEt$



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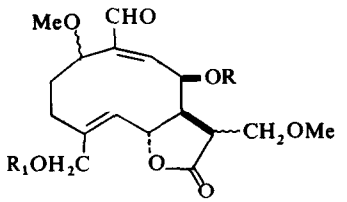
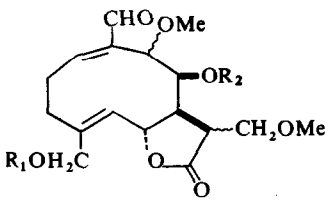
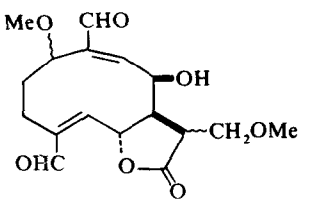


4a $R_1 = R_2 = H$
4b $R_1 = R_2 = Ac$



5a $R_1 = R_2 = H$
5b $R_1 = R_2 = Ac$



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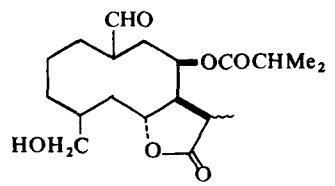

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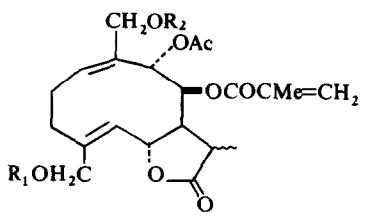
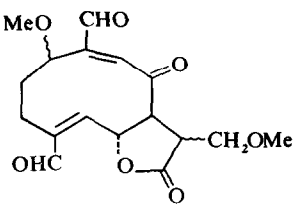


9a $R_1 = R_2 = H$
9b $R_1 = R_2 = Ac$



7

MeOH for 8 hr. The CH_2Cl_2 extract was concd and the residue dissolved in 500 ml MeOH containing 50 ml H_2O. The soln was washed with petrol (bp 10–80°) (3 l.), concd and extracted with $CHCl_3$. The washed and dried extract was evapd to yield a crude gum (18 g) which was chromatographed over 300 g Si gel BDH (60–120 mesh), 150 ml fractions being collected. The column was eluted using the following solvent mixtures: 1–10 (C_6H_6–EtOAc 4:1), 11–20 (C_6H_6–EtOAc 2:1), 21–30 (C_6H_6–EtOAc 1:1), 31–40 (C_6H_6–EtOAc 1:2), 41–50 (C_6H_6–EtOAc 1:4), 51–60 (EtOAc), 61–70 (EtOAc–MeOH 50:1), 71–80 (EtOAc–MeOH 20:1), 81–90 (EtOAc–MeOH 10:1), 91–100 (EtOAc–MeOH 7:1). Since TLC of fractions 24–38 indicated the presence of one major spot, they were combined to give 1.12 g of a gummy material which was further purified by PLC (C_6H_6–EtOAc 1:2) to give pure orientalide (**2a** 0.91 g) as a colourless gum which could not be induced to crystallize: $[\alpha]_D^{25}$ + 41.2° (*c*, 0.034 $CHCl_3$), CD curve (MeOH–$CHCl_3$, *c* 9 mg/ml), $[\theta]_{400}$ 0, $[\theta]_{334}$ − 1350, $[\theta]_{294}$ − 510, $[\theta]_{254}$

– 10040 (last reading). It has IR bands at 3500 (OH), 1780 (conjugated lactone) 1730, 1720 (esters), 1690 (conjugated aldehyde), 1625, 1200, 1180, 1125, 1028 and 980 cm^{-1}, UV strong end absorption (ε_{235} 14800, EtOH). The low resolution MS gave the molecular ion peak at *m/e* 404 and other major peaks at *m/e* 387, 358, 344, 326, 318, 300, 275, 258, 240, 229, 212, 69 (base peak). In the high resolution MS, the molecular ion was not observed. The peak at highest *m/e* corresponded to $C_{17}H_{18}O_6$ due to loss of methacrylic acid (Calc. 318.1102. Found 318.1102). Peaks at *m/e* 300, 275, 258, 240, 229 and 212 corresponded to $C_{17}H_{16}O_5$, $C_{15}H_{15}O_5$, $C_{15}H_{14}O_4$, $C_{15}H_{12}O_3$, $C_{14}H_{13}O_3$ and $C_{14}H_{12}O_2$, respectively. (Calc. for $C_{21}H_{24}O_8$: C, 62.37; H, 5.98. Found: C, 62.15; H, 5.83%).

Preparation of **2b**. Acetylation of **2a** with Ac_2O/Py at room temp. provided **2b** as a gum; IR bands at 1770, 1735, 1720, 1625, 1220, 1130, 1025 and 985 cm^{-1}, low resolution MS *m/e* 446(M^+), 417, 400, 387, 386, 377, 361, 355, 344, 317, 300, 275, 258, 240, 229, 211, 183, 83, 69, 43 (base peak). Calc. for $C_{23}H_{26}O_9$: C, 61.88; H, 5.87. Found: C, 61.64; H, 5.42%).

Hydrolysis of orientalide. A soln of 50 mg of **2a** in 10 ml of MeOH containing 1 ml of 40% aq. KOH was stirred in N_2. After 1 hr, TLC of the reaction mixture indicated that all the starting material had disappeared. The reaction mixture was acidified with dil. HOAc and extracted with $CHCl_3$. The washed and dried extract was evapd and the residue purified by PLC (C_6H_6–EtOAc 1:2). The solid material (20 mg) thus obtained was crystallized from EtOAc to provide **4a**, mp 90–92°; IR bands at 3600 sharp (OH, hydrogen bonded), 3400 br (OH), 1770, 1685, 1625, 1150, 1090, 975 cm^{-1}; significant peaks in the low resolution MS at *m/e* 340 (M^+), 322 ($M^+ - H_2O$), 311 ($M^+ - CHO$), 309 ($M^+ - CH_2OH$), 293 ($M^+ - H_2O - CHO$), 291, 275, 259, 119, 117, 112, 45 (base peak). (Calc. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 60.42; H, 7.38%).

Acetylation of **4a** gave the diacetate **4b** as a gum; IR bands at 1760, 1735, 1690, 1195, 1100, 1025, 980 cm^{-1}; ^1H NMR signals ($CDCl_3$) at 9.48 (–CHO), 6.80 *dbr* (9, H-9), 6.15 *dbr* (9, H-8) 5.08*t* (10.5, H-6), 4.98 *dbr* (10.5, H-5), 4.86 (H-15); 3.4, 3.34 (two methoxyls), 2.16 (two acetates); significant peaks in the MS at *m/e* 424 (M^+), 395 ($M^+ - CHO$), 393 ($M^+ - OMe$), 381 ($M^+ - C_2H_3O$), 365 ($M^+ - C_2H_3O_2$), 364 ($M^+ -$ 364 ($M^+ - C_2H_4O_2$), 321 ($M^+ - C_2H_4O_2 - C_2H_3O$), 304 ($M^+ - 2C_2H_4O_2$), 303 ($M^+ - 2C_2H_4O_2 - H$), 302, 272, 243, 240.

Oxidation of **2a** to **3**. A soln of 25 mg of **2a** in 5 ml spectral grade $CHCl_3$ was stirred at room temp. with 100 mg active MnO_2, the reaction being followed on TLC. After 2 hr the reaction mixture was filtered and the ppt. washed thoroughly with $CHCl_3$. The combined filtrate and washings were evapd and the residue purified by TLC (C_6H_6–EtOAc 1:1) to give **3** as a gum, yield 15 mg; IR bands at 1770, 1735, 1690, 1625, 1220, 1130, 1025, 995 and 960 cm^{-1}; ^1H NMR signals ($CDCl_3$) at 10.15 (H-15), 9.50 (H-14), 6.80 *dd* (9.8, H-1) 6.64 *dd* (9, 1.5, H-8), 5.0 *t* (10.5, H-6), 1.92 (H-4' and acetate); significant peaks in the low resolution MS at *m/e* 402 (M^+), 373 ($M^+ - CHO$), 274 ($M^+ - C_4H_6O_2 - C_2H_2O$), 360 ($M^+ - C_2H_2O$), 316 ($M^+ - C_4H_6O_2$), 313 ($M^+ - C_2H_4O_2 - CHO$), 273 ($M^+ - C_4H_6O_2 - C_2H_2O - H$), 256 ($M^+ - C_4H_6O_2 - C_2H_4O_2$), 245, 227, 119, 117.

Hydrogenation of **2a**. A soln of **2a** (35 mg) in 25 ml EtOH was hydrogenated over 10% Pd/C for 0.5 hr. The major product was separated by PLC to give **8** as a gum, yield 10 mg; IR bands at 3400, 1760, 1725 (br); low resolution MS gave the molecular ion peak at *m/e* 354, other significant peaks at 325 ($M^+ - CHO$), 322 ($M^+ - CH_2OH$), 267 ($M^+ - C_4H_7O_2$), 71 (base peak).

$NaBH_4$ *reduction of* **2a**. A soln of 30 mg of **2a** and 50 mg $NaBH_4$ in 5 ml MeOH was stirred at 0° for 4 hr. The product **9a**, purified by PLC ($CHCl_3$–MeOH, 9:1), was obtained as a gum, IR bands 3600 sharp (hydrogen bonded OH), 3400 br (OH), 1740, 1630, 1220, 450, 1000, 960 cm^{-1}.

Acetylation of **9a** gave **9b** as a gum; IR bands at 1740 (br) 1025, 1225, 1150, 1025, 980 cm^{-1}, significant peaks in the MS *m/e* 492 (M^+) 407 ($M^+ - C_4H_5O_2 - 2C_2H_4O_2$), 227 ($M^+ - C_4H_5O_2 - 3C_2H_4O_2$), 212 ($M^+ - C_4H_5O_2 - 3C_2H_4O_2 - CH_3$).

Oxidation of **4a** to **6**. A soln of 10 mg of **4a** in 5 ml $CHCl_3$ was stirred with 100 mg active MnO_2 for 2 hr. After the usual work-up the residue was purified by PLC (C_6H_6–EtOAc 1:2) and furnished **6** as a gum, yield 6 mg; IR bands at 3400 br (OH), 1780, 1690, 1625, 1150, 1100, 1000, 990 cm^{-1}. The low resolution MS gave significant peaks at *m/e* 338 (M^+), 309 ($M^+ - CHO$) and 307 ($M^+ - CH_2OH$).

Oxidation of **6** *to* **7**. A soln of **7** (6 mg) in 1 ml DMSO was treated with 1 ml Ac_2O and left overnight at room temp. After dilution with ice-cold H_2O it was extracted with $CHCl_3$. The washed and dried extract was evapd and the residue purified by PLC (C_6H_6–EtOAc 1:1) to give **7**, 4 mg as a gum; IR bands at 1780, 1685, 1625, 1600, 1125, 1095, 1010 cm^{-1}; low resolution MS gave significant peaks at *m/e* 337 ($M^+ + 1$), 308 ($M^+ + 1 - CHO$) and 276 ($M^+ - CHO - CH_2OH$).

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