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NEW MELAMPOLIDES AND DARUTIGENOL FROM *SIGESBECKIA ORIENTALIS*

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Key Word Index—*Sigesbeckia orientalis*; Compositae; Heliantheae; Melampodiinae; melampolides; sesquiterpene lactones; darutigenol.

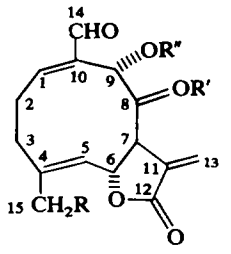
Abstract—Isolation and identification of darutigenol and two new melampolides from *Sigesbeckia orientalis*, in addition to the previously described orientalide and darutoside, are reported.

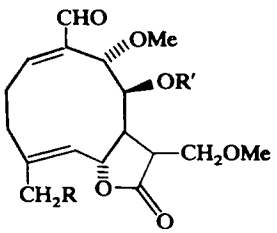
INTRODUCTION

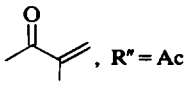
Isolation of the melampolide orientalide (**1a**) from the medicinal plant *Sigesbeckia orientalis* L. has been reported earlier [1]. We now describe isolation of two new melampolides **1b** and **4a** and the diterpene darutigenol (**8a**) from the more polar fractions of the extracts, which also contained the known darutoside [3].

RESULTS AND DISCUSSION

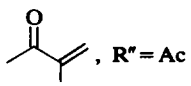
The non-crystalline lactone **1b**, $C_{21}H_{24}O_7$ (high resolution MS) had a ^1H NMR spectrum (see Experimental) essentially superimposable on that of acanthospermal A (**1c**) [4] except for the signals of the two ester side chains which were acetate and methacrylate instead of isobutyrate and α-hydroxyisobutyrate. This was corroborated by the MS (see Experimental). Sequences C-1 through C-3 and C-5 through C-9 were established by spin decoupling in the manner detailed previously [1, 4] as was the fact that the aldehyde function was conjugated with the 1,10-double bond, the latter being *cis* (chemical shift of H-14). Identity of the various coupling constants with those of the acanthospermals and orientalide indicated the stereochemistry shown in the formula. Allocation of the methacrylate to C-8 and the acetate to C-9 was based on analogy to **1c** and **4a** since selective hydrolysis of one of the ester groups could not be affected. Instead, treatment with KOH–MeOH afforded **2a** by solvolytic displacement of the ester on C-9 in the manner previously observed with the acanthospermals and **1a**, concomitant with hydrolysis of the ester on C-8 and addition of methanol to the conjugated lactone. In **2a** attachment of the ring methoxy group to C-9 was verified by acetylation to **2b** and oxidation (DMSO–Ac_2O) to **3**. In the ^1H NMR spectrum of **3**,



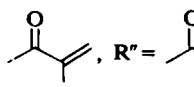
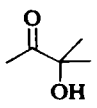


1a R = OH, R' =  , R" = Ac

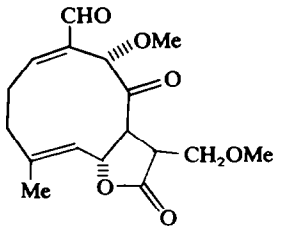
2a R,R' = H

1b R = H, R' =  , R" = Ac

2b R = H, R' = Ac

1c R = H, R' =  , R" = 

2c R = OH, R' = H



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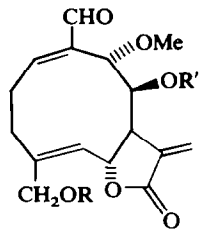
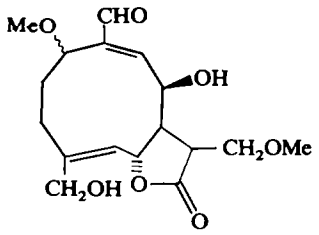


4a R = H, R' = 

4b R = Ac, R' = 



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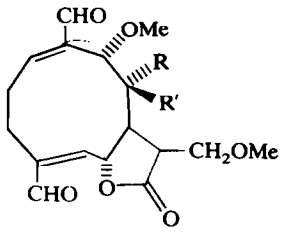
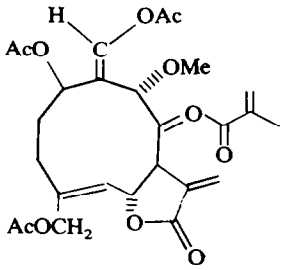


6a R = H, R' = OH
6b R, R' = =O



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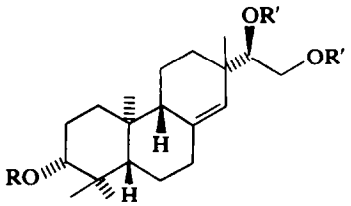
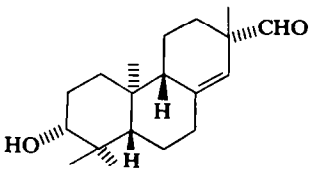


8a R,R' = H
8b R,R' = Ac
8c R,R' = ϕ—C(=O)—
8d R = β-D-glucose, R' = H



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the signal of the proton under the methoxyl group was a sharp singlet at 3.80 ppm and the signal at 7.01 ppm of the proton β to the aldehyde remained coupled to the protons of a methylene group found at 2.72 and 2.49 ppm. Consequently we can dismiss the possibiltity considered in our orientalide paper [1] that in this series solvolysis of the group attached to C-9 may be accompanied by allylic rearrangement. The product from methanolysis of **1a** is therefore **2c**, not **5**.

The second lactone **4a**, mp 208°, $C_{20}H_{24}O_7$, was an analog of orientalide containing a methoxyl group on C-9 (upfield shift of H-9 from 5.3 to 3.8 ppm). Acetylation gave **4b**; hydrolysis with KOH–MeOH yielded **2c** identical with material previously [1] obtained by methanolysis of **1a**. MnO_2 oxidation of **2c** gave the known **6a** [1] which was further oxidized (DMSO–Ac_2O) to **6b**. The ^1H NMR spectrum of the latter confirmed the location of the methoxyl on C-9 and hence the absence of a rearrangement in the methanolysis of **1a**. Exposure of **4a** to BF_3–Ac_2O gave a triacetate which is formulated as the enol acetate **7**.

Darutigenol (**8a**), which has not been isolated previously from *S. orientalis* [2, 3], was identified through conversion to the triacetate (**8b**), tribenzoate (**8c**) and the aldehyde **9**. Its stereochemistry at C-15 has been established recently [5].

EXPERIMENTAL

The extraction of *S. orientalis* has been described [1]. Since fractions 41–90 of the original chromatogram showed several spots on TLC, they were combined (10.9 g) and rechromatographed over 400 g Si gel, fractions being collected as follows: 1–10 (C_6H_6–EtOAc, 1:4), 11–20 (EtOAc), 21–30 (EtOAc–MeOH, 19:1) and 31–40 (EtOAc–MeOH, 9:1) Fractions 4–12 (2.18 g) contained three substances which were separated by PLC (C_6H_6–EtOAc, 1:2). The least polar substance **1b** was a gum (0.55 g) which was not completely pure (*vide infra*) and had IR bands ($CHCl_3$) at 2700 (-CHO), 1775 (lactone), 1730 (esters), 1690 (α, β-unsaturated aldehyde), 1650 and 1140 cm^{-1}: UV strong end absorption (ε_{230} 18 600). ^1H NMR (270 MHz $CDCl_3$): δ 9.48 (J = 2 Hz, H-14), 6.75 (*m*, H-1), 6.75 (*dd*, J = 9, 1.5 Hz, H-8), 6.29 and 5.85(*d*, J = 3 Hz, H-13), 6.04 and 5.60 (*br*, H-3'), 5.33 (*dd*, J = 9, 2 Hz, H-9), 5.10 (*t*, J = 10 Hz, H-6), 4.92 (*br*, J = 10 Hz, H-5), 2.84 and 2.48 (*m*, H-2), 2.65 (*m*, H-7), 2.04 (*br*,

H-15), 1.94 (Ac) and 1.92 (*br*, H-4'). The signals of H-3 were submerged near 1.95 ppm. Additional weak signals indicated the presence of an impurity which could not be removed by PLC. The low resolution MS exhibited significant peaks at *m/e* 388 (M^+), 328 ($M-HOAc$), 31 ($M-C_4H_5O$), 277 ($M^+-C_4H_5O-C_2H_2O$), 259 ($M^+-C_4H_5O-HOAc$), 242 ($M^+-C_4H_6O_2-HOAc$), 213 and 69 (C_4H_5O). (Calc. for $C_{21}H_{24}O_7$: MW, 388.1520. Found: MW(MS), 388.1516).

The next substance **4a** was recrystallized from MeOH, mp 208° (0.42 g). IR bands at 2700, 1760, 1720, 1690 and 1180 cm^{-1}, ^1H NMR (60 MHz): δ 9.60 (*d*, *J* = 2 Hz, H-14), 6.82 (*ddbr*, *J* = 9, 8 Hz, H-1), 6.65 (*dd*, *J* = 9, 1 Hz, H-8), 6.28 (*d*, *J* = 3 Hz, H-13 a), 6.10 (*br*, H-3'), 5.85 (*d*, *J* = 3 Hz, H-13 b), 5.65 (*br*, H-3'), 5.10 (*m*, H-5 and H-6), 4.50 (*br*, H-15), 3.80 (*dd*, *J* = 9, 2 Hz, H-9), 3.18 (OMe), 1.98 (*br*, H-4'). The MS exhibited significant peaks at *m/e* 376 (M^+), 358 (M^+-H_2O), 347 (M^+-HCO), 290 ($M^+-C_4H_6O_2$), 273, 261, 259, 243 and 69 (C_4H_5O). (Calc. for $C_{20}H_{24}O_7$: C, 63.82; H, 6.43. Found: C, 63.56; H, 6.18%).

The most polar substance was identified as darutigenol (**8a**), yield 0.48 g, mp 168°, $[\alpha]_D$ −12° (*c*, 1.024), reported [2] mp 168–170°, $[\alpha]_D$ −11°, MS *m/e*: 322, 305, 304, 291, 286, 273, 271, 261, 227, 187, 173, 135, 120, 109, 107, 105. (Calc. for $C_{20}H_{34}O_3$: C, 74.49; H, 10.63. Found: C, 74.32; H, 10.41%). Gummy triacetate **8b**, MS *m/e* 448, tribenzoate **8c** mp 82°, reported [2, 3] mp 83°. Degradation of **8a** (0.15 g with 0.1 g sodium periodate in MeOH for 12 hr and recrystallization of the crude product from MeOH gave 0.102 g of **9**, mp 118–120°, reported [2, 3] mp 115–120°, MW (MS) 290.

Fractions 25–36 exhibited a single spot on TLC, and were combined and recrystallized from EtOH to give 2.6 g of darutoside (**8d**), mp 250°, $[\alpha]_D$ −35°, reported [2, 3] mp 248–250°, $[\alpha]_D$ −37°, hexaacetate mp 92–94°, reported [2] mp 91–93°. Oxidation of 0.25 g of **8d** in MeOH with 0.20 g of $NaIO_4$ for 12 hr followed by hydrolysis of the crude product with KOH in EtOH and acidification also gave **9**, mp 118–120°.

Reactions of **1b**. A mixture of 0.10 g **1b**, 10 ml MeOH and 0.5 ml 40% KOH was stirred for 3 hr under N_2, acidified with HOAc and extracted with $CHCl_3$. The washed and dried extract was evapd; the residue was purified by PLC (C_6H_6–EtOAc, 1:2) and recrystallized from EtOAc. Yield of **2a** 40 mg, mp 135–140°, IR ν_{max} cm^{-1}: 3500, 1770, 1690 and 1100; UV λ_{max}^{MeOH} nm: 230 (ε 9600), ^1H NMR (60 MHz): δ9.50 (*d*, *J* = 2 Hz, H-14), 6.80 (*dd*, *J* = 9, 8 Hz, H-1), 5.0 (*m*, H-5 and H-6) 3.5–3.95 (H-8, H-9, H-13), 3.40 and 3.25 (OMe), 1.98 (*br*, H-15); MS *m/e*: 324 (M^+), 306, 295, 292, 277, 274, 245, 242, 213. (Calc. for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.81; H, 7.24%).

Acetylation (Ac_2O–Py) of 20 mg **2a** gave 20 mg **2b**, mp 170° (from MeOH). IR bands at 1770, 1720, 1690 and 1160 cm^{-1}; ^1H NMR: δ 9.45 (*d*, *J* = 2 Hz, H-14), 6.70 (*dd*, *J* = 9, 8 Hz, H-1), 6.20 (*dbr*, *J* = 9, 2 Hz, H-8), 5(*m*, H-5 and H-6), 3.6–3.8 (H-9 and H-13), 3.35 and 3.15 (OMe), 2.15 (Ac) and 1.96 (*dbr*, H-15); MS *m/e*: 366 (M^+), 337, 334, 324, 306, 277, 274. (Calc. for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.12; H, 6.95%).

Oxidation of **2a**. A soln of 30 mg **2a** in 1 ml DMSO and 1 ml Ac_2O was kept overnight at room temp, diluted with H_2O and extracted with $CHCl_3$. The washed and dried extract was evapd and the residue purified by PLC to give 20 mg **3**, mp 195–200° after 4 recrystallizations from MeOH. IR ν_{max} cm^{-1}: 2800, 1780, 1720 and 1690; UV strong end absorption (ε_{230} 12 000); ^1H NMR (270 MHz): δ 9.50 (H-14), 7.01 (*ddbr*, *J* = 9, 8 Hz, H-1), 5.16 (*d*, *J* = 10 Hz, H-5), 4.64 (*t*, *J* = 10 Hz, H-6), 3.87 (*dd*, 9.5, 1 Hz) and 3.44 (*dd*, *J* = 9.5, 2 Hz, H-13), 3.87 (H-9), 3.37 and 3.33 (OMe) superimposed on two proton multiplet of H-7 and H-11, 2.72 (*m*, H-2a), 2.49 (*m*, H-2b and H-3a), 2.22 (*t*, *J* = 13 Hz, H-3b), 1.90 (*br*, H-15); MS *m/e*: 322 (M^+), 294, 262, 251, 249, 231. (Calc. for $C_{17}H_{22}O_6$: MW, 322.1415. Found: MW(MS), 322.1414).

Reactions of **4a**. Acetylation of 30 mg **4a** with Ac_2O–Py gave 30 mg **4b** as a gum which had IR bands at 2800, 1770, 1720, 1690 a d 1180 cm^{-1}; ^1H NMR (60 MHz): δ 9.50 (*d*, *J* = 2 Hz, H-14), 6.80 (*dd*, *J* = 9, 8 Hz, H-1), 6.60 (*dd*, *J* = 9, 1 Hz, H-8), 6.26 and 5.88 (*d*, *J* = 3 Hz, H-13), 6.10 and 5.60 (*br*, H-3'), 5.20 (*m*, H-5 and H-6), 4.85 (*br*, H-15), 3.80 (*dd*, *J* = 9, 2 Hz, H-9), 3.15 (OMe), 2.10 (Ac) and 1.98 (*br*, H-4'); MS *m/e*: 418 (M^+), 389, 387, 353, 349, 333, 289, 272, 243, 211 and 69.

A soln of 0.1 g **4a** in 10 ml MeOH was hydrolysed with 40% aq. KOH as described for **1b**. The product (**2c**) was recrystallized from ETOAc and melted at 90–92°, mmp undepressed with material fron hydrolysis of **1a** [1]; ^1H NMR and MS identical. Oxidation of 25 mg **2c** with MnO_2 as previously described [1] gave dialdehyde **6a** as a gum whose ^1H NMR spectrum was not described earlier; signals appeared at δ 10.17 (*br*, H-15), 9.47 (*d*, *J* = 2 Hz, H-14), 6.85 (*dd*, *J* = 9, 8 Hz, H-1), 5.90 *d* (*br*, *J* = 9 Hz, H-5), 4.85 (*dbr*, *J* = 9 Hz, H-6), 3.38 and 3.15 (OMe). Oxidation of 20 mg **6a** with DMSO–Ac_2O as described for **2a** gave 15 mg **6b** as a gum which had IR bands at 2820, 2620, 1780, 1720, 1680, 1625, 1100, 1050 and 1000 cm^{-1}; ^1H NMR; δ 10.23 (H-15), 9.66 (H-14), 7.10 (*dd*, *J* = 9, 8 Hz, H-1), 6.40 (*d*, *J* = 10 Hz, H-5), 5.45 (*dd*, *J* = 10, 9 Hz, H-6), 3.70 (H-9), 3.40 and 3.35 (OMe); MS *m/e*: 336 (M^+), 308, 276, 263 and 245. (Calc. for $C_{17}H_{20}O_7$: MW, 336.1208. Found: MW (MS), 336.1204).

A mixture of 0.04 g **4b**, 2 ml Ac_2O and 5.5 ml BF_3–etherate was left overnight at room temp. poured into cold $NaHCO_3$ soln and extracted with EtOAc. The washed and dried extract was evapd and the residue purified by PLC (C_6H_6–EtOAc, 1:1) to give 25 mg as a gum which had IR bands at 1770, 1720 and 1160 cm^{-1}; ^1H NMR (60 MHz): δ 7.22 (H-14), 6.38 and 5.85 (*d*, *J* = 3 Hz, H-13), 6.1 (overlapping signals of H-1, H-8 and H-3'), 5.65 (*br*, H-3'), 5.28 (*m*, H-5 and H-6), 4.90 (H-15), 3.90 (*d*, *J* = 9 Hz, H-9), 3.15 (OMe), 2.20, 2.15, 2.15 (Ac), 2.0 (*br*, H-4'); MS *m/e*: 520 (M^+), 489, 461, 434, 387, 359, 349, 333, 289, 273, 272, 241, 213, 212, 211 and 69.

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