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Sesquiterpene lactones and a neolignan from *Hyaloseris andrade-limae*

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Abstract

Aerial parts of *Hyaloseris andrade-limae* furnished the eudesmanolides ivasperin and isoivasperin, the guaianolide 14-hydroxypseudoivalin, the peptide aurentiamide acetate and a new neolignan hyaloserin.

Keywords: *Hysaloseris andrade-limae*; Mutisieae; Asteraceae; Eudesmanolides; Guaianolide; Neolignan

1. Subject and source

Aerial parts of *Hyaloseris andrade-limae* Cristobal and Cabrera (1982) were collected on February 10, 1998 at Sierras de Guasayán, San Pedro de Guasayán, Santiago del Estero province, Argentina. The plant material was identified by Lic. Alberto Slanis. A voucher specimen (Slanis and Riscala s/n LIL 600980) has been deposited in the Herbarium Instituto Miguel Lillo, Tucumán, Argentina.

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2. Previous work

Hyaloseris Griseb. (Mutisieae, Asteraceae) is a genus of seven Bolivian and Argentine species which according to Espinar (1973) and Hansen (1991) includes the monotypic genus *Dinoseris* as *Hyaloseris salicifolia* (Griseb.) Hieron. Previous work on the genus is limited to *H. salicifolia* whose extraction furnished the eudesmanolide **2c** together with common acetylenes (Bohlmann et al., 1979) and subsequently (De Riscala et al., 2000) the eudesmanolide ivasperin (**1a**) and its 6-*O*-acetyl-β-D-glucoside **1b**.

3. Present work

3.1. General procedures

For separation of mixtures, HPLC with a differential refractometer detector was used. The columns employed were a Beckman C-18 (column A) and a Beckman C-8 (column B), both 10 × 150 mm and 5 μ particle size. Retention times were measured from the solvent peak. ^{1}H NMR spectra were run on Varian Inova 500 MHz NMR spectrometer. Mass spectra were run on a JEOL MS Route 600 H instrument.

3.2. Extraction and isolation of constituents

Flowers and leaves (1.5 kg) of *H. andrade-limae* were extracted with $CHCl_3$ (2 × 6 l) at room temperature for 7 days. The extract was evaporated at reduced pressure (40 °C) to give 88.25 g of crude extract which was suspended in EtOH (760 ml) at 60 °C, diluted with H_2O (570 ml) and extracted successively with 3 × 800 ml of hexane and 3 × 700 ml of $CHCl_3$. Evaporation of the $CHCl_3$ extracts at reduced pressure furnished 14.98 g of residue a portion of which (10 g) was chromatographed by CC over Si gel (410 g, 230–400 mesh) using $CHCl_3$ with increasing amounts of EtOAc (0–100%), 87 fractions being collected which were grouped on the basis of their TLC profiles and monitored by IR spectroscopy. Fractions showing γ-lactone absorption were processed by RP-HPLC. Fractions 14–17 (279 mg) were combined and a portion (102 mg) was processed by HPLC using column A (MeOH–H_2O 65:35, 1.7 ml min^{-1}) to give 26 mg of a sesquiterpene lactone mixture (gum, R_t 11.7 min) and 6.2 mg of aurentiamide acetate (**5**, mp 185°, R_t 34.9 min) identified by ^{1}H NMR spectrometry and comparison with authentic material (Catalán et al., 2003). Fraction 18 (22 mg) on HPLC (column A, MeOH–H_2O 55:45) gave 9.5 mg of a mixture of **5** and an unidentified sesquiterpene lactone (R_t 25 min). Fraction 19 (112 mg) on HPLC as above gave mixtures and more **5** (3.8 mg). Fractions 27 and 28 (190 mg) were combined, a portion (62 mg) on HPLC (column A, MeOH–H_2O 55:45, 2 ml min^{-1}) afforded 28 mg of somewhat contaminated **3b** as a gum (R_t 3 min). Fractions 37 and 38 (60 mg) were combined; HPLC (column B, MeOH–H_2O 1:1, 1.7 ml min^{-1}) gave 7.3 mg of a gummy fraction whose ^{1}H NMR spectrum indicated the presence of impure **4**, a previously unreported substance, with identifiable signals at δ 9.47 (d, $J = 1$ Hz,

H-15), 6.67 (t, $J = 8.7$ Hz, H-3), 6.21 (d, $J = 3.2$ Hz, H-13a), 5.51 (d, $J = 2.7$ Hz, H-13b), 5.36 (brdd, $J = 8.7$, 6.7 Hz, H-9), 4.23 (brd, $J \sim 9$ Hz, H-6), 2.87 (m, H-7), 2.67 (c, H-8a, H-2a), 2.53 (c, H-2b, H-5), 2.04 (brq, $J = 12$ Hz, H-8b), 1.69 (brs, H-14). The low resolution CIMS exhibited the $M + H^+$ ion of this compound at m/z 245. This fraction was followed by 2.1 mg of a mixture and 10.2 mg of **2a**.

Fractions 39–43 (363 mg) consisted of crude **2a**. Recrystallization from EtOAc furnished pure isoivasperin (**2a**), mp 162–163° (211 mg). Fractions 44–47 (524 mg) were combined. A portion (100 mg) on HPLC as above furnished mixtures of **1a** and **2a** followed by pure **1a**, 55 mg, mp 161–162°). Fractions 48–50 (277 mg) were combined, HPLC (column B, MeOH–H_2O 1:1, 1.7 ml min^{-1}) afforded 22 mg of a mixture, 86 mg of **1a** (R_t 11 min) and unidentified material. Rechromatography of the mixture (column B, MeOH–H_2O 45:55) gave 3.5 mg of **2a** (R_t 20.5 min) and 1.9 mg of neolignan **6** (R_t 27.6 min). Fractions 51–87 (4.51 g) did not exhibit lactone absorption and were not investigated further.

3.3. Identification of constituents

Ivasperin (**1a**). Mp 161–162°, was identified by comparison with authentic material (Herz et al., 1964). Our 500 MHz NMR spectral data in $CDCl_3$ differ somewhat from the 300 MHz spectrum in DMSO-d_6 reported by Riscala et al. (2000), δ 0.80 (3H, d, 0.7 Hz, H-14), 1.43 (ddd, $J = 14$, 14, 12.4, Hz, H-6a) 1.47 (dd, $J = 15.5$, 4.7 Hz, H-9a) 1.58 (brs, OH), 1.79 (ddd, $J = 14$, 7, 2.8 Hz, H-6b) 1.86 (brd, $J = 12.5$ Hz, H-5) 2.07 (brt, $J = 12$ Hz, H-3a), 2.19 and 2.33 (both br, OH), 2.62 (dd, $J = 12$, 5.5 Hz, H-3b), 2.64 (dd, $J = 1.6$, 15.5 Hz, H-9b), 2.95 (dd, ddd, $J = 12$, 7, 5, 1, 1 Hz, H-7), 3.11 (d, $J = 9$ Hz, H-1), 3.56 (ddd, $J = 12$, 9, 5.5 Hz, H-2), 4.53 (td, $J = 5$, 5, 1.8 Hz, H-8) 4.59 (q, $J \sim 1.2$ Hz, H-15a), 4.90 (q, $J \sim 1.2$ Hz, H-15b), 5.59 (d, $J = 0.9$ Hz, H-13a), 6.13 (d, $J = 1.1$ Hz, H-13b).

Isoivasperin (**2a**). Mp 162–163°, was the new $\Delta^{4,5}$ isomer of ivasperin as shown by the MS, the ^1H and the ^{13}C NMR spectra; HRCIMS 265.1445 (48.0), 247.1327 (57.6), (calculated for $C_{15}H_{20}O_4 + H^+$, 265.1440, for $C_{15}H_{20}O_4 - H_2O + H^+$, 247.1334); ^1H and NMR spectrum (500 MHz, $CDCl_3$) δ 6.21 (d, $J = 2$ Hz, H-13a), 5.64 (d, $J = 1.3$ Hz, H-13b), 5.27 (d, $J = 4$ Hz, H-6), 4.83 (dt, $J = 6.7$, 3.3 Hz, H-8), 3.93 (ddd, $J = 12$, 9.5, 4.5 Hz, H-2), 3.57 (dddd, $J = 6.7$, 4, 2, 1.3 Hz, H-7), 3.00 (d, $J = 9.5$ Hz, H-1), 2.59 (dd, $J = 14.8$, 3 Hz, H-9a), 2.58 (ddd, $J = 6$, 7, 1.5 Hz, H-4), 1.87 (ddd, $J = 12.7$, 4.5, 1.5 Hz, H-3a), 1.55 (dd, $J = 14.8$, 3 Hz, H-9b), 1.54 (ddd, $J = 12.7$, 12, 6 Hz, H-3b), 1.19 (s, 3p, H-14), 1.14 (d, $J = 7$ Hz, 3p, H-15), ^{13}C NMR spectrum (50 MHz, $CDCl_3$) δ 84.41 (C-1), 67.08 (C-2), 39.30 (C-3), 39.32 (C-4), 146.34 (C-5), 121.31 (C-6), 39.30 (C-7), 75.06 (C-8), 38.00 (C-9), 37.83 (C-10), 129.27 (C-11), 170.08 (C-12), 122.20 (C-13), 23.49 (C-14), 23.10 (C-15). The new lactone is the desacetyl derivative of **2b** isolated earlier from *Eriophyllum lanatum* (Bohlmann et al., 1981), a member of a genus most recently placed in subtribe Baerinae of Helenieae (Karis and Ryding, 1994).

Lactone (**3b**), obtained as a gum, could not be completely freed of the presence of a very small amount of contaminant; however, ^1H NMR spectrometry indicated the presence of a guaianolide lactonized towards C-8 which contained a primary

hydroxyl group on C-14, a C-1, C-10 double bond, a tertiary hydroxyl under the methyl group on C-4 and a *cis*-lactone closed toward C-8 with the usual conjugated exocyclic methylene group on C-11. Hence, the substance was 14-hydroxypseudoivalin (**3b**), a derivative of pseudoivalin (**3a**), the latter first isolated (Herz et al., 1964; Anderson et al., 1975) from *Iva microcephala* (Heliantheae, Gaillardiinae); HRCIMS 265.1436 (19.2), 247.1341 (100) (calculated for $C_{15}H_{20}O_4 - H_2O + H^+$, 265.1440, for $C_{15}H_{18}O_3 + H$, 247.1341, ^{1}H NMR spectral data (500 MHz, $CDCl_3$) δ 1.28 (3p, s, H-15), 2.11 (c, H-6a, b, H-9b), 2.17 (brdd, 13, 1.4 Hz, 2H) and 2.71 (dd, $J = 10.3$, 4.6 Hz, 1H)—these signals corresponding to H-2a,b and 3a, 2.94 (brdd, 14, 4.3 Hz, H-9a), 3.03 (m, H-7), 4.24 and 4.13 (each d, $J = 11.7$ Hz, H-14a,b), 4.64 (ddd, $J = 11.5$, 4.3 4.3 Hz, H-8), 5.57 (d, $J = 2.6$ Hz, H-13b), 6.31 (d, $J = 3$ Hz, H-13a).

Substance (**6**), obtained only in very small amount, was a new neolignan. The MS and ^{1}H NMR spectrum indicted that it differed from a relatively recently described analog 3′, 4-di-*O*-methylcedrusin (Lemière et al., 1995) in substitution of the pendant aromatic ring which now carried a hydroxyl group at the 4′ and two methoxyls at the 3′- and 5′-positions, and lacked the methoxyl on the dihydrobenzofuran moiety. We have named the substance hyaloserin.

3-[2-(3,5-dimethoxy-4-hydroxyphenyl)-3-hydroxymethyl-2,3-dihydro-1-benzofuran-5yl]-propan-1-ol (hyaloserin) (**6**). Gum; HRCIMS m/z 360.1577 (calculated for $C_{20}H_{24}O_6$ 360.1573); EIMS (70 eV) m/z 360 (45), 342 (100); ^{1}H NMR spectral data (500 MHz, $CDCl_3$) δ 1.87 (2H, approximate dt, J = 14, 7.5, 6.5 Hz, H-8′a,b), 2.66 (2H, dd, $J = 8$, 7.5 Hz, H-7′a,b), 3.59 (brq, $J = 6.5$ Hz, H-8), 3.68 (2H, t, $J = 6.5$ Hz, H-9′a,b) 3.85 and 3.87 (each s, 3H, -OMe), 3.88 (dd, $J = 11$, 4.7 Hz, H-9b) 3.96 (dd, $J = 11$, 6 Hz, H-9a), 5.53 (d, $J = 7.5$ Hz, H-7), 6.66 (s, 2H, H-2, 6), 6.86 (d, $J = 8$ Hz, H-3′), 6.89 (dd, $J = 8$, 1.8 Hz, H-2′), 6.92 brd, $J = 1.8$ Hz, H-6′).

4. Chemotaxonomic significance

The chemistry of *H. andrade-limae* while slightly more diverse does not differ significantly from that of the only other previously studied member of the genus, *H. salicifolia*, which seems to support the arguments in favor of merging *Dinoseris* with *Hyaloseris* (Espinar, 1973; Hansen, 1991; Bremer, 1994). On the other hand the chemistry of these two species seems somewhat unusual compared with the chemistry of other genera ordinarily placed in subtribe Mutisiinae of Mutiseae.

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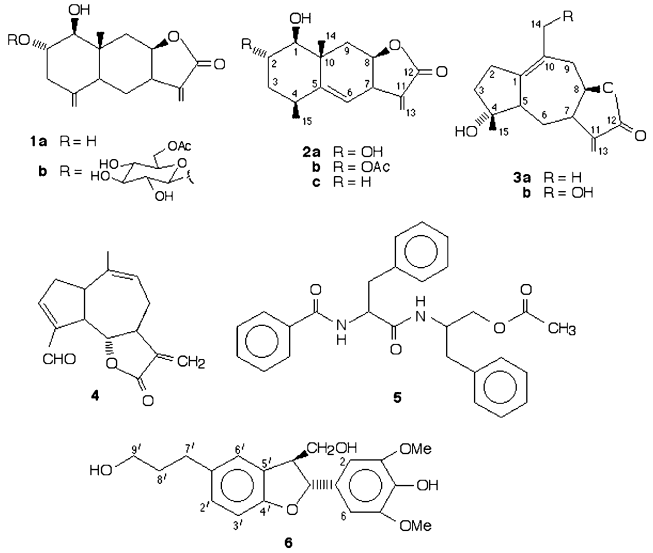

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