Manoyl Oxide α-Arabinopyranoside and Grindelic Acid Diterpenoids from Grindelia integrifolia

Ahmed A. Ahmed,*† Ahmed A. Mahmoud,† Usama M. Ahmed,‡ Ashraf A. El-Bassuony,† Mohamed H. Abd El-Razk,§ Paul W. Pare,‡ and Joe Karchesy§

Department of Chemistry, Faculty of Science, El-Minia University, El-Minia 61519, Egypt, Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409, and Department of Forest Products, Oregon State University, Corvallis, Oregon 97331

Received June 6, 2001

Two new manoyl oxide-α-arabinopyranoside diterpenoids, 15-hydroxy-13-epi-manoyl oxide-14-O-α-L-arabinopyranoside (tarapacol-14-O-α-L-arabinopyranopyranoside) (1) and 15-acetoxy-13-epi-manoyl oxide-14-O-α-L-arabinopyranoside (tarapacol-15-acetate-14-O-α-L-arabinopyranoside) (2), as well as a new grindelic acid derivative, 19-hydroxygrindelic acid (3), together with five known diterpenoids (tarapacol, tarapacanol A, grindelic acid, methyl grindeloate, 3β-hydroxygrindelic acid, 4) were isolated from the aerial parts of Grindelia integrifolia. The structures of 1–3 were elucidated by spectral data analysis.

The New World genus Grindelia (family Asteraceae, tribe Astereae, subtribe Soldiginae) is characterized by the abundant production of resinous exudates that cover the surfaces of the leaves, stems, and involucres of the flower head.1 Earlier work on this genus revealed that diterpenoid acids of the labdane type, grindelic acid derivatives, are characteristic secondary metabolites.2–9 Some manoyl oxide derivatives have been isolated from G. tarapcana.10 Recently, we reported the isolation and characterization of three new grindelic and norgrindelic acid derivatives from G. nana.11 In a continuation of our studies, we have now investigated the aerial parts of G. integrifolia DC., which has not been previously analyzed chemically. We report herein on the isolation and structure characterization of three new diterpenoids, including two manoyl oxide arabinopyranoside derivatives, 15-hydroxy-13-epi-manoyl oxide-14-O-α-L-arabinopyranoside (tarapacol-14-O-α-L-arabinopyranoside) (1) and 15-acetoxy-13-epi-manoyl oxide-14-O-α-L-arabinopyranoside (tarapacol-15-acetate-14-O-α-L-arabinopyranoside) (2), and a new grindelic acid derivative, 19-hydroxygrindelic acid (3). The known compounds, tarapacol,10 tarapacanol A,10 grindelic acid,2,12,13 methyl grindeloate,2,12,13 and 3β-hydroxygrindelic acid (4),8 were also isolated and identified by comparison of their MS and 1H and 13C NMR spectral data with those reported in the literature.

High-resolution CIMS provided a quasi-molecular ion peak [M + H]+ at m/z 457.3165, corresponding to a molecular formula C29H45O7, for compound 1. The presence of an arabinosyl moiety in 1 was inferred from the CIMS fragment ion peak at m/z 325 [M + H – arabinosyl]+ and from the typical 1H NMR signals which appeared at δ 4.24 (1H, d, J = 7.9 Hz, H-1′), 3.75 (1H, dd, J = 9.2, 7.9 Hz, H-2′), 3.66 (1H, dd, J = 9.2, 3.0 Hz, H-3′), 3.90 (1H, brs, H-4′), 4.08 (1H, dd, J = 14.0, 2.0 Hz, H-5a′), and 3.59 (1H, d, J = 14.0 Hz, H-5b′). These proton signals showed correlations in the 1H–13C COSY NMR spectrum with carbon resonances at δ 106.8 d (C-1′), 72.9 d (C-2′), 73.9 d (C-3′), 68.3 d (C-4′), and 66.5 t (C-5′), respectively, which are comparable to standard values reported in the literature.14–16 The coupling constant of the anomeric proton (7.9 Hz) and the small couplings between the H-3′ and H-4′ (3.0 Hz) and the H-4′ and H-5a′ protons (2.0 Hz) indicated the α-configuration of a l-arabinopyranoside unit.16,17 In agreement with the molecular formula, the 13C NMR spectrum displayed, in addition to the five signals of the arabinosyl moiety, 20 other carbon signals, suggesting the presence of a diterpene core unit. DEPT experiments indicated that these 20 signals corresponded to five methyl groups, eight methylene groups, three methine groups, and four quaternary carbons (Table 1). The structure of the...
A diterpene was elucidated in part by 1H and 13C NMR analysis (Table 1). These spectral data were quite similar to those exhibited by the major compound, tarapacol (14,15-dihydroxy-13-epi-manoyl oxide). In addition to the signals that were already assigned to the sugar component, the 1H NMR spectrum contained five tertiary methyl signals at δ 0.78 (H-19), 0.79 (H-20), 0.84 (H-18), 1.17 (H-16), and 1.23 (H-17), integrating for three protons each within the manoyl oxide framework. Furthermore, an oxygenated proton at δ 3.45 (dd, J = 8.5, 3.0 Hz, H-14) showed correlations in the 1H–1H COSY NMR spectrum with methylene hydrogens of a primary alcohol at δ 3.39 (dd, J = 12.0, 8.5 Hz, H-15a) and 3.48 (dd, J = 12.0, 3.0 Hz, H-15b). The assignment of all protons in 1 and their connectivities to adjacent protons and carbons was established from the results of 2D 1H–1H COSY, 1H–13C COSY, and HMBC NMR experiments. Accordingly, compound 1 was an arabinopyranoside derivative of 14,15-dihydroxy-13-epi-manoyl oxide. To establish the location of the arabinosyl moiety on the manoyl oxide skeleton, the 13C NMR data were compared between compound 1 and tarapacol, a manoyl oxide diterpene devoid of the sugar component. In tarapacol, C-14 and C-15 appeared at δ 75.8 and 63.6, respectively, while the values observed for 1 were 95.3 (d, C-14) and 61.6 (t, C-15), indicating that the arabinosyl moiety was attached to C-14. This was supported by the HMBC correlations (Table 2) of the anomeric proton H-1′ (δ 4.24) to C-14 of the manoyl oxide skeleton and H-14 (δ 3.45) to C-1′ of the sugar moiety. Finally, acid hydrolysis of 1 gave an aglycon having R₁ and 1H and 13C NMR spectral data identical to those of tarapacol. The sugar fraction gave a positive optical rotation, +101° (c 0.12, H2O), in accordance with l-arabinopyranoside. Thus, the structure of 1 was assigned as 15-hydroxy-13-epi-manoyl oxide-14-O-α-L-arabinopyranoside (tarapacol-14-O-α-L-arabinopyranoside).

Compound 2 showed an [M + H]⁺ peak at m/z 499 and a molecular formula of C27H46O8 as deduced by HRCIMS (m/z 499.3261). The 1H NMR spectrum was very similar to that of tarapacol-14-O-α-L-arabinopyranoside (1). However, the methylene protons of the primary alcohol (H-15a and H-15b) were shifted downfield (δ 3.97 and 4.10, respectively) in 2 compared with those at δ 3.39 and 3.48 in 1. In addition, the spectrum contained a new methyl signal (δ 2.06, s), corresponding to an acetyl group. The placement of the acetyl group at C-15 was deduced from HMBC measurements, which correlated H-15a (δ 3.97) with the acetyl carbonyl group (δ 171.0). The 13C NMR spectrum was similar to 1 except for two new signals at δ 21.4 (q) and 171.0 (s), which were typical of an acetate group. The products of the acid hydrolysis of 2 were similar to those of 1. Therefore, 2 was identified as 15-acetoxy-13-epi-manoyl oxide-14-O-α-L-arabinopyranoside (tarapacol-15-acetate-14-O-α-L-arabinopyranoside).

The HRCIMS of compound 3 exhibited a [M + H]⁺ peak at m/z 337.2374 in accordance with the molecular formula C26H34O4 (calc m/z 337.2378). This molecular formula along with similarities in the 1H and 13C NMR spectra indicated that this compound might be a hydroxy derivative of grindelic acid. However, differences included the replacement of the methyl group signal (Me-19) with a pair of doublets at δ 3.81 and 3.54 (d, J = 11.0 Hz) in 3. Therefore, a hydroxyl group was placed at C-19. The proton signals at δ 3.81 and 3.54 (H-19a and H-19b) showed cross-peaks to C-3, C-4, and C-18, and the methine proton at δ 1.81 (H-5) showed cross-peaks to C-4, C-10, C-19, and C-20, confirming this observation in the HMBC spectrum. The relative stereochemistry at C-4 was given by the difference NOE NMR spectrum, which showed enhancements between H-19a and Me-20, as well as between H-5a and Me-18. Therefore, 3 was assigned as 19-hydroxygrindelic acid. It is interesting to note that the structure of compound 3 has been reported from Grindelia paludosa. However, 1D and 2D NMR measurements including 13C NMR, 1H–13C COSY, and HMBC spectra, which have not been performed previously, were inconsistent with the previous data of this structure and may warrant revision of the earlier structure.

**Experimental Section**

**General Experimental Procedures.** Optical rotation measurements were made on a Perkin-Elmer FT spectrometer. IR spectra (oily films) were taken on a Perkin-Elmer 1366 spectrum. 1H and 13C NMR, DEPT, 1H–1H COSY, 1H–13C COSY, and HMBC (delay, 173.9 µs) spectra were measured on a JEOL JNM-GX-500 spectrometer, with TMS as an internal standard. Mass spectra were recorded with a J EOL JMS-D300 mass spectrometer using direct inlet electron impact ionization (70 eV). TLC: precoated silica gel 60F254.
plates (Merck); preparative TLC: silica gel PF 254 (Merck, 200 × 200 × 0.25 mm); column chromatography: silica gel type 60 (Merck, 200). Plant Material. G. integrifolia was collected in July 1996 at Port Townsend, WA. A voucher specimen (No. 187558) has been deposited at the Department of Forest Products, Oregon State University, Corvallis, OR.

Extraction and Isolation. The air-dried, powdered aerial parts of G. integrifolia (500 g) were extracted with CH3Cl2—MeOH (1:1) at room temperature, and the extract was concentrated to obtain 105 g of residue. The residue was prefractionated by column chromatography on Si gel eluting with petroleum ether (60–80 °C), followed by a gradient of petroleum ether—EtO2 (up to 100% EtO2) and EtO2—MeOH, into five fractions: fraction 1 (petroleum ether—EtO2, 3:1), fraction 2 (petroleum ether—EtO2, 1:1), fraction 3 (petroleum ether—EtO2, 1:3), fraction 4 (EtO2, 100%), and fraction 5 (EtO2—MeOH, 9:1). Fractions 1 and 2 were separated on a Si gel column eluted with n-hexane—CH2Cl2 (2:1) to give fractions 1-A and 1-B. Fraction 1-A was further purified on a Sephadex LH-20 column eluted with n-hexane—CH2Cl2—MeOH (4:7.0:5) to afford methyl grindelolate12,13 (200 mg). Fraction 1-B was repeatedly chromatographed on a Sephadex LH-20 column eluted with n-hexane—CH2Cl2—MeOH (4:7.0:5) to give grindelic acid2,12,13 (500 mg). Fraction 3 was further purified on a Sephadex LH-20 column eluted with n-hexane—CH2Cl2—MeOH (4:7.0:5), to give tarapacol 19-Hydroxygrindelic acid (3): colorless oil; [α]D 25 0° (CHCl3, c 0.17); 1R (NaCl) νmax 3445, 2925, 1455, 1380 cm−1; 1H NMR spectral data (CDCl3, 400 MHz) (Table 1; CIMS (positive ion) m/z 499 [M + H]+ (30), 367 [M + H – arabinosyl]10 (28), 349 [M + H – arabinosyl – H2O]+ (31), 307 (45), 289 (39), 271 (45), 263 (20), 191 (30); HRMS m/z 499.3261 (calcld for C27H45O8, 499.3270).

19-Hydroxygrindelic acid (3): colorless oil; [α]D 25 +44° (CHCl3, c 0.17); 1R (NaCl) νmax 3445, 2925, 1455, 1380 cm−1; 1H NMR spectral data (CDCl3, 499 MHz) (1H, br s, H-7), 3.81 (1H, d, J = 11.0 Hz, H-19a), 3.54 (1H, d, J = 11.0 Hz, H-19b), 2.69 (1H, d, J = 15.8 Hz, H-14a), 2.57 (1H, d, J = 15.8 Hz, H-14b), 1.81 (1H, m, H-5), 1.76 (3H, br s, H-17), 1.25 (3H, s, H-17), 1.49 (3H, s, H-16), 0.98 (3H, s, H-18), 0.81 (3H, s, H-20); HMBC H-5 (C-4, C-10, C-19, C-20), H-7 (C-8, C-5), H14a (C-13, C-15, C-16), H-16 (C-12, C-13, C-14, H-17, C-17, C-8, C-9), H-18 (C-4, C-8, C-19), H-19a and H-19b (C-3, C-4, C-18), H-20 (C-1, C-5, C-9, C-10); NOESY Me-18 (H-5α, H-19a); 13C NMR spectral data, see Table 1; CIMS (positive ion) m/z 337 [M + H]+ (80), 319 [M + H – H2O]+ (40), 288 [M + H – CH2O + H2O]− (85), 223 (70), 177 (25), 153 (35), 135 (20); HRMS m/z 337.2374 (calcld for C25H45O8, 337.2378).

Acknowledgment. This work was financially supported at Texas Tech University by the Robert A. Welch Foundation (D-1478). A.A.A. thanks the Alexander von Humboldt-Stiftung for financial support for the HPLC instrument.

References and Notes