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7-*O*-METHYLHORMINONE AND OTHER CYTOTOXIC DITERPENE QUINONES FROM *LEPECHINIA BULLATA*

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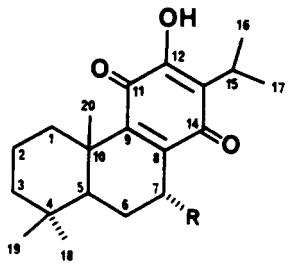
ABSTRACT.—Three cytotoxic diterpene quinones, 6,7-dehydroroyleanone [**1**], horminone [**2**], and 7-*O*-methylhorminone [**3**], were isolated from an MeOH extract of *Lepechinia bullata* after bioassay-directed fractionation. Compound **3** is a new compound whose structure was determined through spectroscopic analysis.

Continuing our search for potential antitumor agents from plant extracts, we examined an MeOH extract of the above-ground parts of *Lepechinia bullata* (Kunth) Epling (Labiatae) for its cytotoxic activity in cultured KB (nasopharyngeal carcinoma) and P-388 (murine leukemia) cells. It was found that the crude extract was active in the latter system (ED_{50} = 14.5 μg/ml), while the KB cells were less sensitive (ED_{50} = 40.5 μg/ml).

The bioactive MeOH extract was then separated by solvent extraction and bioassay-directed chromatography to afford three cytotoxic diterpene quinones, namely, 6,7-dehydroroyleanone [**1**], horminone [**2**], and 7-*O*-methylhorminone [**3**]. The last compound is a new natural product, whose presence in a plant sample directly extracted with $CHCl_3$ was confirmed by tlc.



1 R=H; $\Delta^{6,7}$
2 R=OH
3 R=OMe
4 R=H

6,7-Dehydroroyleanone [**1**] and horminone [**2**] were initially recognized by their spectroscopic properties (^1H and ^{13}C nmr, uv, ir, and ms) (1,2). Identities were then confirmed by direct comparison with authentic samples (mmp, [α]D, co-tlc). 6,7-Dehydroroyleanone [**1**] has been isolated from plants of the Labiatae family, including *Plectranthus* (1,3), *Rosmarinus* (4), *Coleus* (5), *Salvia* (2,6,7), as well as *Inula* (Compositae) (8) and *Podocarpus* (Podocarpaceae) (9). Horminone [**2**] was obtained previously from *Rosmarinus* (4), *Hormium* (10), *Plectranthus* (1), *Coleus* (5), and *Salvia* (4,6, 11–13) species. Both compounds are reported here for the first time from *L. bullata*, which has not been previously investigated. In the literature, a few other *Lepechinia* species have been analyzed for their essential oil compositions; they include *Lepechinia chalepensis*

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(14), *Lepechinia floribunda* (15), *Lepechinia speciosa* (16), *Lepechinia graveolens* (17), and *Lepechinia salviae* (18). Diterpenes and triterpenes have also been isolated from two species, *Lepechinia chamaedryoides* (19) and *Lepechinia glomerata* (20).

The third compound **3**, isolated as yellow needles, mp 126–128°, showed many spectroscopic properties similar to those of horminone [**2**], justifying a royleanone-type structure. The mass spectrum of **3** displayed a molecular ion at *m/z* 346, 14 amu higher than that of **2** ($[M]^+$ 332), revealed as an additional methyl group on a hydroxyl function by the presence of a methoxyl signal in both the ^{1}H- and ^{13}C-nmr spectra (δ 3.45 and 57.3 ppm, respectively). The compound was, therefore, most likely a methyl ether derivative of **2** at either C-7 or C-12. In the literature, 12-*O*-methylhorminone has been reported as a horminone derivative prepared by a methylation reaction (1), while the 7-*O*-methyl analogue is hitherto unknown. Careful comparison of the ^{1}H- and ^{13}C-nmr spectra of **2** and **3** showed that the most significant difference in their chemical shift values was found for H-7 and C-7, besides the presence of an additional methoxyl signal in **3**. Thus, the methoxyl group caused the C-7 to shift downfield to δ 70.8 ppm (from δ 63.2 in compound **2**), and concurrently, the H-7 signal moved upfield to δ 4.32 from δ 4.73. Such nmr results are consistent with a methoxyl substituent replacing the 7-OH of horminone. Further evidence to exclude the possibility of a 12-*O*-methyl structure was obtained from the uv spectra of **3**, in which the absorption at 411 nm exhibited a significant bathochromic shift on the addition of NaOH, indicating that a quinonoid hydroxyl group (12-OH) was present. Similar uv shifts have been observed in diterpene quinones bearing a quinonoid hydroxyl function (21). Finally, direct comparison of a reference sample of 12-*O*-methylhorminone with **3** in several tlc systems showed them to be different.

Compound **3** was, therefore, assigned the structure 7-*O*-methylhorminone. The stereochemistry at C-7 was determined from the ^{1}H-nmr evidence. Kupchan *et al.* (22,23) have compared the H-7β signal of horminone with the H-7α of taxoquinone, its 7-epimer. They concluded that, at 60 MHz, the H-7α appeared as a multiplet with $W_{1/2} = 20$ Hz whereas the H-7β signal was a broad singlet with a $W_{1/2} = 8$ Hz. In this respect, the H-7 of **3**, measured at 300 MHz, was observed as a doublet of doublets with $J = 2$ and 4 Hz, consistent with an β orientation of the proton. The methoxyl group is thus α as depicted in structure **3**.

Assignment of the ^{1}H nmr spectrum of **3** was assisted by the performance of homonuclear correlation (COSY) and heteronuclear correlation (HETCOR) experiments. Among the eight methylene protons in the molecule, four were observed as discernible signals. Thus, a deshielded signal at δ 2.68 ppm (ddd, $J = 4, 4, 11$ Hz) was assigned to H-1β, and a broad doublet at δ 2.04 ppm ($J = 12$ Hz) to H-6α. The signal for H-6β was found at δ 1.35 ppm as a ddd ($J = 4, 12, 13$ Hz), and that for H-2β was discerned at δ 1.70 ppm as a multiplet ($J = 4, 4, 14, 14, 14$ Hz). The rest of the methylene protons overlapped with other signals and were not readily analyzable. Protons of compound **2** were then assigned by comparison with those of **3**.

With the proton assignments available, the protonated carbons could be assigned from the HETCOR spectrum. In order to aid the assignments of quaternary carbons, selective INEPT experiments (24) were performed with horminone [**2**]. In the *J*-modulated selective INEPT experiment, a particular proton is irradiated with a soft pulse resulting in magnetization transfer and a selective enhancement of carbon atoms three bonds away from the irradiated proton. Thus, polarization transfer from H-7 ($^3J = 6$ Hz) enhanced a carbonyl resonance (189.0 ppm), an aromatic carbon resonance (147.8 ppm), and a protonated resonance (45.7 ppm, C-5), of which the former two could be assigned to C-14 and C-9, respectively. As a result, the two carbonyl carbons (C-11 and C-14), as well as the two quaternary aromatic carbons C-8 and C-9, could be unam-

biguously determined. In addition, C-13 (124.1 ppm) was assigned through irradiation of Me-16/17 ($^3J = 6$ Hz). Because the proton resonances of both 20-Me and H-2α happened to fall into the same range of chemical shifts as the Me-16/17 groups, several other enhanced signals were observed when Me-16/17 was irradiated. These enhancements confirmed the assignments of C-1, C-4, C-5, C-9, and C-10. Complete assignments of the ^{13}C-nmr spectrum of 7-*O*-methylhorminone [**3**] were then made by comparison with those of **2**.

To date, only a few diterpene quinones have been found to display antitumor activity. Both royleanone [**4**] and taxoquinone, the 7-epimer of **2**, were shown to be devoid of activity in the KB system (22,23). Belonging to the same class of chemical structure, compounds **1–3** did not exhibit any significant cytotoxicity against KB cells in the present study ($ED_{50} = 5.7$, 20.2, and 13.0 μg/ml, respectively). On the other hand, all isolates inhibited the growth of P-388 cells ($ED_{50} = 1.6$, 4.6, and 4.8 μg/ml for **1**, **2**, and **3**, respectively), although compounds **2** and **3** were only marginally active according to the guidelines of the National Cancer Institute (25). They represent the first examples of diterpene quinones of the royleanone type to be found cytotoxic against mammalian tumor cells, although horminone [**2**] has previously been reported to inhibit the growth of *Trypanosoma cruzi* (26). It is also worth noting that, in both the KB and P-388 systems, a 6,7-saturated structure bearing a 7-oxygenated substituent seemed to decrease the activity, when compared to that of the 6,7-dehydro compound **1**. Because it is logical to speculate that the activity of these molecules is due to the substituents associated with the C ring, this modulation of activity through transformation at position 7 is of general interest in terms of structure-activity relationships.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Uv spectra were obtained with a Beckman DU-7 spectrophotometer, and the ir spectra on a Nicolet MX-1 Ft-ir instrument. ^{1}H- and 2D-nmr spectra were recorded with a Varian XL-300 spectrometer operating at 299.94 MHz for protons and 75.44 MHz for carbons, while the ^{13}C-nmr and selective INEPT spectra were measured on a Nicolet NMC-360 instrument operating at 90.8 MHz. Low-resolution eims were obtained with a Varian MAT-112S mass spectrometer at 70 eV.

PLANT MATERIAL.—The above-ground parts of *L. bullata* were collected in Colombia in May 1976, by members of the USDA. Voucher specimens have been deposited at the National Herbarium, Washington, D.C.

FRACTIONATION AND CHROMATOGRAPHY.—The dried, powdered plant material (1.5 kg) was exhaustively extracted with 95% MeOH to yield a syrupy residue after removal of the solvent in vacuo. The crude extract was washed with petroleum ether, followed by partition between $CHCl_3$ and aqueous MeOH. The $CHCl_3$ fraction (61 g) was chromatographed over Si gel (3 kg), using $CHCl_3$ as eluting agent. A total of 50 fractions (500 ml each) were collected, analyzed by tlc, and combined on the basis of similar chromatograms.

ISOLATION OF 6,7-DEHYDROROYLEANONE [**1**].—Fractions 6–19, on standing in the cold room overnight, deposited an orange precipitate, which was collected by filtration. It was purified by preparative tlc using $CHCl_3$ as solvent, followed by crystallization to give orange crystals (62 mg; 0.004% yield from dried material), shown to be 6,7-dehydroroyleanone [**1**] by spectral analysis and direct comparison with an authentic sample.

ISOLATION OF HORMINONE [**2**].—The major component in fractions 47–49, following repeated chromatography, afforded horminone [**2**] as a yellow crystalline compound (362 mg, 0.024% yield): ^{1}H nmr (300 MHz, $CDCl_3$) δ 4.73 (d, $J = 4$ Hz, H-7β), 3.16 (septet, $J = 7$ Hz, H-15), 3.04 (s, D_2O exchangable, 7-OH), 2.69 (ddd, $J = 4$, 4, 12 Hz, H-1β), 1.96 (d, $J = 11$ Hz, H-6α), 1.72 (m, H-2β), 1.55 (hidden, H-5), 1.5–1.7 (m, H-3α,β), 1.4–1.5 (m, H-6β), 1.2–1.3 (hidden, H-2α), 1.21 (d, $J = 7$ Hz, Me-16), 1.22 (d, $J = 7$ Hz, Me-17), 1.22 (s, Me-20), 1.1–1.2 (m, H-1α), 0.98 (s, Me-18), 0.90 (s, Me-19) ppm; ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 189.0 (C-14), 183.8 (C-11), 151.1 (C-12), 147.8 (C-9), 143.1 (C-8), 124.1 (C-13), 63.2 (C-7), 45.7 (C-5), 41.0 (C-3), 39.1 (C-4), 35.7 (C-1), 33.1 (C-18), 33.0 (C-10), 25.7 (C-6), 23.9 (C-15), 21.7 (C-19), 19.8 (C-17), 19.7 (C-16), 18.8 (C-2), 18.3 (C-20) ppm.

ISOLATION OF 7-*O*-METHYLHORMINONE [**3**].—Fractions 31–42 were combined and rechromatographed to yield a yellowish-brown semicrystalline product, which was further purified by preparative tlc ($CHCl_3$ as solvent) and crystallizations to afford yellow crystals (75 mg; 0.005% yield): mp 126–128°; uv (EtOH) λ max 271, 411 nm; uv (EtOH + NaOH) λ max 274, 524 nm; ir (KBr) ν max 3376, 1646, 1638 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 4.31 (dd, J = 2, 4 Hz, H-7β), 3.45 (s, 7-OMe), 3.18 (septet, J = 7 Hz, H-15), 2.68 (ddd, J = 4, 4, 11 Hz, H-1β), 2.04 (d, J = 12 Hz, H-6α), 1.70 (m, H-2β), 1.57 (hidden, H-5α), 1.4–1.6 (m, H-3α,β), 1.35 (ddd, J = 4, 12, 13 Hz, H-6β), 1.2–1.3 (m, H-2α), 1.22 (d, J = 7 Hz, Me-17), 1.21 (s, Me-20), 1.1–1.2 (m, H-1α), 1.19 (d, J = 7 Hz, Me-16), 0.95 (s, Me-18), 0.91 (s, Me-19) ppm; ^{13}C nmr (90.8 MHz, $CDCl_3$) δ 186.4 (C-14), 184.1 (C-11), 150.6 (C-12), 147.8 (C-9), 141.4 (C-8), 124.7 (C-13), 70.7 (C-7), 57.3 (7-OMe), 45.5 (C-5), 41.0 (C-3), 39.2 (C-4), 35.7 (C-1), 33.0 (C-10), 33.0 (C-18), 24.2 (C-15), 22.1 (C-6), 21.9 (C-19), 19.9 (C-17), 19.7 (C-16), 18.8 (C-2), 18.5 (C-20) ppm; ms *m/z* (rel. int.) $[M]^+$ 346 (100%), 331 (58), 314 (34), 299 (23), 271 (6), 245 (14), 231 (7), 218 (7), 203 (5), 187 (4), 109 (8), 83 (17), 69 (17), 55 (27), 43 (38), 41 (37).

BIOASSAYS FOR CYTOTOXICITY.—Cytotoxic activity of the extracts and compounds was tested in KB and P-388 cell cultures (25) according to standard procedures as described previously (27,28).

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