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A DITERPENE XYLOSIDE FROM THE RESINOUS EXUDATE OF *HAPLOPAPPUS DIPLOPAPPUS*

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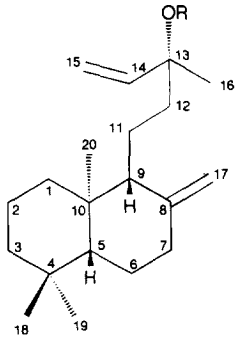
Key Word Index—*Haplopappus diplopappus*; Compositae; diterpene; labdane xyloside.

Abstract—From the resinous exudate of *Haplopappus diplopappus*, a new labdane glycoside named 13-*O*-β-xylopyranosyl-*ent*-manool was obtained. The structure was deduced by chemical and spectroscopic evidence (COSY, HMQC, NOE and MS-FAB). The known compound *ent*-manool was also isolated.

INTRODUCTION

In this paper we describe the isolation and characterization of a new labdane glycoside, named 13-*O*-β-xylopyranosyl-*ent*-manool (**2**), from the resinous exudate of *Haplopappus diplopappus* Remy. The aerial parts of this plant have been used in folk medicine in Chile [1, 2], but there are no reports about its chemical composition. Compound **2** shows an unusual sugar location and its structure was elucidated by use of 1 and 2D NMR (COSY, HMQC, NOE), MS-FAB data, and hydrolysis. In addition, the known labdane diterpene *ent*-manool (**1**) was also obtained [3, 4].



1 R= H
2 R= β-D-Xylopyranoside

RESULTS AND DISCUSSION

The dichloromethane extract obtained from the resinous exudate of *H. diplopappus* gave the labdane diterpenes **1** and **2**.

The FAB mass spectrum of **2** exhibited the molecular ion peak at *m/z* 445 $[M+Na]^+$, indicating the molecular mass of 422. In the low resolution mass spectrum the base peak appeared at *m/z* 273 corresponding to the loss of a terminal pentose ($C_5H_9O_5$) unit. Acetylation of **2** resulted in the incorporation of three acetate groups (δ_H 2.04, 2.02, 2.02) and acid hydrolysis gave D-xylose as the sugar component. Enzymic hydrolysis with β-D-xylosidase afforded an aglycone whose spectral data were identical to those of *ent*-manool (**1**).

The ^1H and ^{13}C NMR spectra of **2** showed the signals expected for xylose bound to the rest of the molecule by glycosidation as β-D-xylopyranoside [anomeric signal at δ_H 4.47 (*d*, $J=6.0$ Hz) and δ_C 97.7]. As regards the aglycone, the most significant signals were four singlets for methyl groups at δ_H 1.36, 0.86, 0.79 and 0.66, and five olefinic protons, three typical for a vinilic group at δ_H 5.78 (*dd*, $J=17.5$, 10.9 Hz), 5.25 (*d*, $J=9.8$ Hz), 5.20 (*d*, $J=17.4$ Hz), and two representing an exo methylene group at δ_H 4.80 and 4.48.

The structure was confirmed by homo- and heteronuclear COSY, DEPT and NOE difference experiments. Thus, all the spectral data were consistent with **2** having the structure of 13-hydroxy-labda-8(17),14-diene-13-*O*-β-xylopyranoside.

The antimicrobial activity of **2** was tested against seven Gram (+) bacteria, four Gram (−) bacteria and two yeast. The compound was inactive against the yeast *Saccharomyces cereviseiae* and *Candida albicans*, and against the Gram (−) bacteria *Escherichia coli*. It showed only slight activity against *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis*, *B. antharcis*, *Staphylococcus aureus* and *S. epidermidis*, and medium activity against *Bacillus pumilus*, *Micrococcus luteus* and *M. flavus*. These results are in agreement with some of the pharmacological properties associated with the resinous exudates of *Haplopappus* spp. [1, 2].

EXPERIMENTAL

General. Mps: uncorr.; ^{1}H and ^{13}C NMR: $CDCl_3$, TMS as int. standard; 2D ^{1}H–^{1}H and ^{13}C–^{1}H correlation spectra were obtained using standard Brucker software. FAB-MS: Kratos MS-50 MS equipped with a Kratos FAB source; sample dissolved in a glycerol matrix with NaCl as additive; EIMS: direct inlet, 70 eV; CC: Merck silica gel (60–230 mesh) and Woelm N (grade IV) neutral alumina; TLC: silica gel GF-254.

Plant material. Specimens of *H. diplopappus* Remy were collected in El Colorado (33°19′S, 70°14′W) in February 1990, near the city of Santiago, Chile. The plant was identified by Mélica Muñoz, chief of the Botanical Department of the Natural History Museum in Santiago, Chile. A voucher specimen is deposited in the herbarium of the Natural History Museum in Santiago, Chile.

Extraction and separation. The resinous exudate of *H. diplopappus* was extracted by immersion of the just collected plant material in CH_2Cl_2 for 15–20 sec at room temp. The CH_2Cl_2 extract (7.7 g, 0.75% dry wt) was purified by CC (silica gel) using a hexane–CH_2Cl_2–MeOH step gradient. Compound **1** (60 mg) was sepd from the 1st frs by prep. TLC (CH_2Cl_2–hexane). The polar frs were further sepd by CC (neutral alumina) to give, after prep. TLC ($CHCl_3$–MeOH), **2** (70 mg).

ent-*Manool* (**1**). Gum; $[\alpha]_D^{20}$ −31.1° ($CHCl_3$; *c* 0.2) (ref. [4] −29.5°); ^{1}H NMR (250 MHz, $CDCl_3$): δ0.67 (3H, *s*, 10-Me), 0.80 (3H, *s*, 4β-Me), 0.87 (3H, *s*, 4α-Me), 1.27 (3H, *s*, 13-Me), 1.99 (1H, *m*, H-12), 2.37 (1H, *m*, H-12′), 4.48 (1H, *s*, H-17), 4.81 (1H, *s*, H-17′), 5.06 (1H, *dd*, J = 1.3, 10.7 Hz, H-15), 5.22 (1H, *dd*, J = 1.3, 17.3 Hz, H-15′), 5.91 (1H, *dd*, J = 10.7, 17.4 Hz, H-14); EIMS *m/z* (rel. int.): 290 $[M]^+$ (0.3), 272 $[M-H_2O]^+$ (7), 257 (25), 137 (51), 81 (84), 43 (100); molecular formula $C_{20}H_{34}O$ $[M]^+$ (calcd 290.26000; HRMS found 290.26095).

13-O-β-D-*Xylopyranosyl*-ent-13-*hydroxylabda*-8(17), 14-*diene* (**2**). Gum; $[\alpha]_D^{20}$ −40.1° ($CHCl_3$; *c* 1.0); Positive FAB-MS *m/z* (rel. int.): 468 $[M + 2Na]^+$ (5), 445 $[M + Na]^+$(100); EIMS *m/z* (rel. int.): 273 $[M - sugar]^+$ (29), 272 (100), 149 $[sugar]^+$ (30), 257 (47), 191 (30), 81 (100); ^{1}H NMR (250 MHz, $CDCl_3$): δ0.66 (3H, *s*, 10-Me), 0.79 (3H, *s*, 4β-Me), 0.86 (3H, *s*, 4α-Me), 1.36 (3H, *s*, 13-Me), 1.95 (1H, *m*, H-12), 2.35 (1H, *m*, H-12′), 3.28 (1H, *dd*, J = 8.4, 11.7 Hz, H-5′$_{ax}$), 3.37 (1H, *dd*, J = 6.5, 7.5 Hz, H-2′), 3.56 (1H, *t*, J = 7.7 Hz, H-3′), 3.69 (1H, *ddd*, J = 4.4, 7.7, 7.7 Hz, H-4′), 4.0 (1H, *dd*, J = 4.3, 11.9 Hz, H-5′$_{eq}$), 4.47 (1H, *d*, J = 6 Hz, H-1′), 4.48 (1H, *d*, J = 1 Hz, H-17), 4.80 (1H, *d*, J = 1 Hz, H-17′), 5.20 (1H, *d*, J = 17.4 Hz, H-15), 5.25 (1H, *d*, J = 9.8 Hz, H-15′), 5.78 (1H, *dd*, J = 10.9, 17.5 Hz, H-14); ^{13}C NMR (62.83 MHz, $CDCl_3$): δ 14.4 (*q*, C-20), 17.6 (*t*, C-11), 19.3 (*t*, C-2), 21.7 (*q*, C-18), 22.5 (*q*, C-16), 24.4 (*t*, C-6), 33.5 (*q*, C-19), 33.6 (*s*, C-4), 38.3 (*t*, C-7), 39.0 (*t*, C-1), 39.8 (*s*, C-10), 40.9 (*t*, C-12), 42.2 (*t*, C-3), 55.6 (*d*, H-5), 57.4 (*d*, C-9), 64.2 (*t*, C-5′), 69.7 (*d*, C-4′), 72.8 (*d*, C-2′), 75.1 (*d*, C-3′), 91.1 (*s*, C-13), 97.7 (*d*, C-1′), 106.4 (*t*, C-17), 116.0 (*t*, C-15), 142.2 (*d*, C-14), 148.8 (*s*, C-8).

13-O-[3′,4′,5′-*tri*-O-*acetyl*-β-D-*xylopyranosyl*]-ent-13-*hydroxylabda*-8(17), 14-*diene*. A soln of **2** (6 mg) in Ac_2O–pyridine (1:1, 5 ml) was maintained at room temp. for 10 hr. The soln was extracted with $CHCl_3$, the extract washed with H_2O (10 ml) and concd to give the triacetate; needles, mp 114–116° (EtOH–H_2O); $[\alpha]_D^{20}$ −43.4° ($CHCl_3$; *c* 1.06); IR ν_{max}^{KBr} cm^{-1}: 1752, 1234 (OAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ0.64 (3H, *s*, 20-Me), 0.78 (3H, *s*, 4β-Me), 0.85 (3H, *s*, 4α-Me), 1.30 (3H, *s*, 13-Me), 1.92 (1H, *m*, H-12), 2.02 (6H, *s*, 2 × OAc), 2.04 (3H, *s*, OAc), 3.26 (1H, *dd*, J = 9.6, 11.6 Hz, H-5′$_{ax}$), 4.06 (1H, *dd*, J = 5.4, 11.7 Hz, H-5′$_{eq}$), 4.43 (1H, *s*, H-17), 4.54 (1H, *d*, J = 7.4 Hz, H-1′), 4.76 (1H, *s*, H-17′), 4.93 (2H, *m*, H-2′, H-4′), 5.14 (1H, *t*, J = 9.0 Hz, H-3′), 5.17 (1H, *d*, J = 17.7 Hz, H-15), 5.23 (1H, *d*, J = 10.7 Hz, H-15′), 5.72 (1H, *dd*, J = 10.9, 17.6 Hz, H-14).

Acid hydrolysis of **2**. Compound **2** (50 mg) was dissolved in 5 ml MeOH and 5 ml 10% HCl. The mixt. was stirred at room temp. for 3 hr; the solvent was evapd and MeOH was added and evapd several times until most of the HCl was eliminated. The remaining residue was diluted with H_2O (5 ml) and the soln neutralized (pH 7.0) with basic resin (Zeo-Karb 215). The suspension was filtered and extracted with CH_2Cl_2; the CH_2Cl_2 extract afforded decomposed aglycone mixt. The H_2O layer was evapd to dryness yielding xylose (co-PC). Final purification was by prep. PC using the system *n*-BuOH–EtO–H_2O (4:9:1) affording pure D-(+)-xylose, $[\alpha]_D^{30}$ +17.4° (H_2O; *c*0.25).

Enzymic hydrolysis of **2**. Compound **2** (50 mg) and β-D-xylosidase (5 U, sigma X 5375) in 3.5 M $(NH_4)_2SO_4$–50 mM sodium acetate, pH 5.2, was incubated at 25° overnight. The reaction mixt. was extracted with CH_2Cl_2 and taken to dryness to give the aglycone in quantitative yield. ^{1}H NMR and MS spectra of the aglycone were identical to those of *ent*-manool (**1**). $[\alpha]_D^{30}$ −31.3° ($CHCl_3$; *c* 0.1).

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