Pergamon 0031-9422(95)00109-3 *Phytochemistry*, Vol. 39, No. 6, pp. 1489–1491, 1995

0031-9422/95 $9.50 + 0.00

DITERPENE ACETYLXYLOSIDES FROM THE EXUDATE OF *CONYZA LINEARIS*

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(*Received* 6 *September* 1994)

Key Word Index—*Conyza linearis*; Compositae; diterpene acetylxyloside; exudate.

Abstract—Two new labdane acetylxylosides, ent-manool-13-*O*-β-D-4′-acetylxylopyranoside and ent-manool-13-*O*-β-D-3′-acetylxylopyranoside were obtained from the exudate of *Conyza linearis*. The structures were deduced from chemical and spectroscopic evidence.

INTRODUCTION

The resinous exudates of several plants of arid and semiarid regions of northern and central Chile have been shown to contain uncommon components. Acylated flavonoids have been reported in the exudates of *Gnaphalium robustum* [1] and *Heliotropium stenophyllum* [2], a rearranged geranyl aromatic derivative in the exudate of *H. filifolium* [3], C-18 and C-19 nor-labdanes in the exudate of *Haplopappus velutinus* [4], and a diterpene xyloside in the exudate of *H. diplopappus* [5]. We describe here the isolation and structural determination of two new diterpene acetyl xylosides, ent-manool-13-*O*-β-D-4′-acetylxylopyranoside (**1**) and ent-manool-13-*O*-β-D-3′-acetylxylopyranoside (**2**), from the exudate of *Conyza linearis* DC.

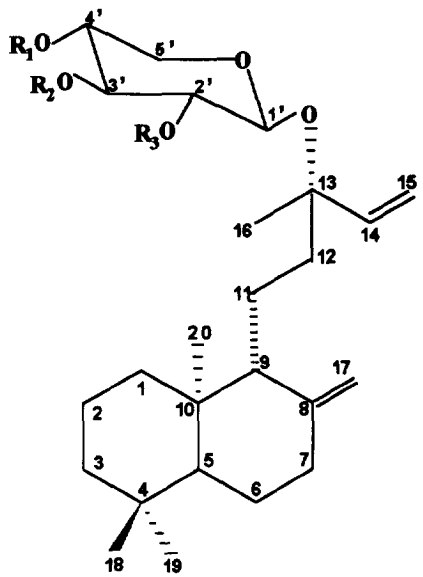
RESULTS AND DISCUSSION

The dichloromethane washing of fresh aerial parts of *C. linearis* gave the labdane diterpenes **1** and **2**.

The mass spectrum of **1** exhibited a small but reproducible $[M]^+$ peak at *m/z* 464 consistent with the molecular formula $C_{27}H_{44}O_6$, and a peak at *m/z* 273 corresponding to the loss of a terminal acetyl pentose ($C_7H_{11}O_6$) unit.

The ^{13}C NMR spectrum confirmed the presence of 27 carbon atoms and was very similar to the spectrum of ent-manool-13-*O*-β-D-xylopyranoside (**4**) previously isolated from *H. diplopappus* [5], but showing two extra carbon resonances which were assigned to an acetyl group (Table 1).

The downfield shift of the C-5′ and C-3′ resonances of 3.7 and 3.5 ppm, respectively, with regard to the spectrum of the unacetylated compound suggested that the acetoxy chain was attached to C-4′; this was confirmed by the downfield multiplet for H-4′ at δ_H4.83 (Table 2) and on the basis of spin decoupling experiments



	R_1	R_2	R_3
1	Ac	H	H
2	H	Ac	H
3	Ac	Ac	Ac
4	H	H	H

(360 MHz). Thus irradiation of the anomeric proton (*d*, δ4.59) collapsed a broad triplet at δ3.48 (H-2′) into a doublet. Irradiation at the latter frequency collapsed not only the anomeric signal into a singlet, but also a broad triplet at δ3.75 (H-3′) into a doublet. Irradiation at the H-3′ frequency collapsed the signal for H-2′ into a doublet and sharpened the H-4′ multiplet at δ4.83.

Table 1. ^{13}C NMR data of compound **1** (100.6 MHz, in $CDCl_3$, with TMS as int. standard)*

C	1†	4‡
1	39.0	39.0
2	19.4	19.3
3	42.1	42.2
4	33.5	33.6
5	55.5	55.6
6	24.4	24.4
7	38.3	38.3
8	148.6	148.6
9	57.3	57.4
10	39.8	39.8
11	17.6	17.6
12	40.9	40.9
13	82.3	81.8
14	141.8	142.2
15	116.2	116.0
16	22.5	22.6
17	106.3	106.4
18	33.5	33.6
19	21.7	21.7
20	14.5	14.4
Xylopyranosyl		
1′	97.1	97.7
2′	72.1	72.8
3′	71.6	75.1
4′	71.3	69.9
5′	60.6	64.3
Acetyl		
1″	170.0	—
2″	20.9	—

*Degree of protonation was obtained by DEPT heteronuclear multipulse programs.

†The assignments were obtained by ^{13}C–^{1}H correlation.

‡From ref. [5].

Irradiation at the H-4′ frequency collapsed the broad triplet at δ3.75 (H-3′) into a doublet and the double doublets at δ4.14 (Hb-5′) and 3.35 (Ha-5′) into doublets. Finally, unequivocal assignments of the ^{13}C resonances of **1** were obtained from ^{13}C–^{1}H correlations. Acid hydrolysis showed that the sugar was D-(+)-xylose. The $J_{1'2'}$ = value (5.5 Hz) of **1** suggested a diaxial relationship between H-1′ and H-2′, and β configuration of the anomeric carbon. Enzymatic hydrolysis with β-xylosidase gave the pure aglycone, identified as ent-manool [6, 7]. Thus, **1** is ent-manool-13-*O*-β-D-4′-acetylxylopyranoside.

The mass spectrum of **2** exhibited a small but reproducible $[M]^+$ peak at *m/z* 464 consistent with the molecular formula $C_{27}H_{44}O_6$, and peak at *m/z* 273 corresponding to the loss of a terminal acetyl pentose ($C_7H_{11}O_6$) unit. The ^{1}H NMR spectrum was also very similar to the spectrum of **1** (Table 2), showing a downfield shift of the H-3′ resonance from δ3.75 to 4.82 and an upfield shift of the H-4′ resonance from δ4.83 to 3.81. These results suggested that the acetyl side chain in **2** was attached at C-3′; this was confirmed, as for **1**, on the basis of spin decoupling experiments (at 360 MHz). Thus, **2** was ent-manool-13-*O*-β-D-3′-acetylxylopyranoside.

Acetylation of **1** and **2** with acetic anhydride in pyridine yielded the same triacetyl derivative **3**, identical in all respects (mp, $[\alpha]_D$, ^{1}H NMR, FTIR) with the triacetyl derivative of ent-manool-13-*O*-β-D-3′-acetylxylopyranoside (**4**) [5], confirming that all three compounds have the same stereochemistry.

EXPERIMENTAL

Conyza linearis DC. was collected during the flowering season, October 1990, in Lo Prado Pass, (33° 10′ S, 71° W), near the city of Santiago, Chile. A voucher specimen is deposited in the Herbarium of the Natural History Museum, Santiago, Chile (Voucher SGO 106000). The exudate was obtained in the field by dipping the fresh plant in cold CH_2Cl_2 for 15–20 sec. The extracts were transported to the laboratory at 0° and concentrated to afford a sticky residue (7.5 g, 1.5% of the dry wt of the plant). Part of the extract (3 g) was fractionated by CC on silica gel, using $CHCl_3$ containing increasing amounts of MeOH. The fractions were monitored by TLC on silica gel using the systems hexane–EtOAC (2:1), $CHCl_3$–MeOH (19:1) and $CHCl_3$–MeOH (49:1), spraying with 33% H_2SO_4 and heating to 120°. Final purification was by prep. TLC using the systems hexane–EtOAc–MeOH (20:30:3) and $CHCl_3$–MeOH (49:1).

ent-Manool-13-O-β-D-4′-*acetylxylopyranoside* (**1**). Gum; 80 mg; $[\alpha]_D^{20}$ −20.7° ($CHCl_3$; *c* 0.018), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580, 3500, 1042 (OH), 1635 (C=C), 1750, 1220 ($MeCO_2R$); EI-MS 70 eV, *m/z* (rel. int.): 464 $[M]^+$ (0.04), 273 (11) [M − acetylxylosyl]$^+$, 272 (14) [M − acetylxylose]$^+$, 204 (11), 189 (7), 137 (100); ^{1}H NMR (Table 2); ^{13}C NMR: Table 1.

ent-Manool-13-O-β-D-3′-*acetylxylopyranoside* (**2**). Gum; 36 mg; $[\alpha]_D^{17}$ −52.4° ($CHCl_3$; *c* 0.019), $\nu_{max}^{CCl_4}$ cm^{-1}: 3575, 3500, 1041 (OH), 1652 (C=C), 1749, 1222 ($MeCO_2R$); EI-MS 70 eV, *m/z* (rel. int.): 464 $[M]^+$ (0.02), 273 (5) [M − acetylxylosyl]$^+$, 272 (5) [M − acetylxylose]$^+$, 204 (8), 137 (49); ^{1}H NMR: Table 2.

Acetylation of compounds **1** *and* **2**. Compounds **1** and **2** were acetylated with Ac_2O–pyridine in the usual manner to give the same triacetate **3**. $[\alpha]_D^{23}$ −51.6° ($CHCl_3$; *c* 0.055), IR ν_{max} cm^{-1}: 1640 (C=C), 1760, 1225 ($MeCO_2R$); EI-MS 70 eV, *m/z* (rel. int.): 548 $[M]^+$ (0.02), 534 [M − Me_3]$^+$ (0.06), 273 [M − triacetylxylosyl]$^+$ (10), 272 [M − triacetylxylose]$^+$ (17), 204 (9), 189 (4), 137 (33), 43 (100).

Acid hydrolysis of compounds **1** *and* **2**. The compounds (15 mg of each) were dissolved in 5 ml MeOH and 5 ml 10% HCl. The mixtures were stirred at room temp. for 3 hr. The solvent was then evapd and MeOH added and evapd several times until most of the HCl was eliminated. The remaining residues were diluted with H_2O (5 ml) and the solns were neutralized (pH 7.0) with basic ion exchange resin (Zeo-Karb 215). The suspensions were fil-

Table 2. ^{1}H NMR spectral data of compounds **1–3** (**1** and **2**, 360 MHz; **3**, 200 MHz, in $CDCl_3$, TMS as int. standard)*

H	1	2	3	4†
14	5.08 *dd* (11, 17)	5.79	5.70	5.79 *dd* (10, 17)
15a	5.21 *dd* (1, 17)	5.22	5.15	5.19 *br d* (17)
15b	5.26 *dd* (1, 17)	5.26	5.21	5.24 *br d* (10)
16	1.36	1.36	1.29	1.34
17a	4.47 *br s*	4.50	4.42	4.49 *br s*
17b	4.81 *br s*	4.80	4.79	4.80 *br s*
18	0.87	0.87	0.83	0.86
19	0.80	0.80	0.76	0.79
20	0.66	0.66	0.63	0.65
1′	4.59 *d* (5.5)	4.42 *d* (7)	4.52	4.39 (7)
2′	3.48 *br t* (5)	3.47 *dd* (7, 9)	5.10 *m*[a]	3.34 *br t* (7)
3′	3.75 *br t* (7)	4.82 *br t* (9)	4.90 *m*[a]	3.49 *br t* (7)
4′	4.83 *m*	3.81 *ddd* (5, 9, 10)	4.90 *m*[a]	3.65 *m*
5a′	3.35 *dd* (7, 12)	3.25 *dd* (10, 12)	3.24	3.21 *br t* (10)
5b′	4.14 *dd* (4, 12)	3.99 *dd* (5, 12)	4.04	3.93 *dd* (4, 11)
Acetyl				
	2.1 *s* (3H)	2.18 (3H)	1.99 (3H)	—
			2.02 (6H)	—

*Multiplicities and coupling constants (in parentheses) are not repeated if they are identical with those in the preceding column.

†From ref. [5].

[a]These signals might be interchangeable.

tered and extracted with CH_2Cl_2; the CH_2Cl_2 extracts afforded decomposed aglycone material. The H_2O layers were evapd to dryness yielding xylose (co-PC). Final purification was by prep. PC using the system *n*-BuOH–EtOH–H_2O (4:1:1). This gave pure (+)-xylose, $[\alpha]_D^{31}$ 17.1° (H_2O; *c* 0.25).

Enzymic hydrolysis of compound **1**. The compound (30 mg) and β-D-xylosidase (5 units, Sigma X 5375) in 3.5 M $(NH_4)_2SO_4$, 50 mM sodium acetate, pH 5.2, were incubated at 25° overnight. The reaction mixture was then extracted with CH_2Cl_2 and taken to dryness to give the aglycone in quantitative yield, as a gum; $[\alpha]_D^{30}$ − 30.3° ($CHCl_3$; *c* 0.1) (lit [7] − 29.5°). ^{1}H NMR and MS: indentical to those of ent-manool.

Acknowledgements—Financial support for this work from FONDECYT and from DICYT (Universidad de Santiago de Chile) is gratefully acknowledged.

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