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LINALOOL AND CINEOLE TYPE GLUCOSIDES FROM *CUNILA SPICATA**

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Key Word Index—*Cunila spicata*; Lamiaceae; Poejo; monoterpenetriol; monoterpeneglucosides.

Abstract—The leaves of *Cunila spicata* yielded a monoterpenetriol and six glycosidic terpenoids derived from linalool, hydroxylated linalool and 1,8-cineole: 3,7-dimethyl-oct-1-ene-3,6,7-triol, linalool-*O*-β-D-glucopyranoside, 3,7 dimethyl-octa-1,6-diene-3,8-diol-3-*O*-β-D-glucopyranoside as well as 3,7-dimethyl-octa-1,5-diene-3,7-diol-3-*O*-β-D-glucopyranoside, 3,7-dimethyl-octa-1,7-diene-3,6-diol-7-*O*-β-D-glucopyranoside, 3,7-dimethyl-oct-1-ene-3,6,7-triol-6-*O*-β-D-glucopyranoside and (1*S*,4*R*,6*R*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-*O*-β-glucopyranoside. The structures of the glucosides were established by chemical and spectroscopic methods especially high field NMR techniques.

INTRODUCTION

The aerial parts of different *Cunila* species, called Poejo, are used in traditional Brazilian medicine against febrile illnesses of the bronchial tract [1]. However, little is known about the chemical constituents of the genus *Cunila*. In a previous paper Hartmann and I reported on the isolation and structural elucidation of five terpene glucosides from *Cunila spicata* L. leaves [2]. In my continuing study on the glycosidic constituents of this Lamiaceae, I examined further the ethyl acetate and the *n*-butanol-soluble portions of *C. spicata*. Column chromatography of these hydrophilic portions yielded six glucosides of linalool (**2**), hydroxylated linalool (**3**, **4**/**5** and **6**), and hydroxylated 1,8-cineole (**7**) as well as a monoterpenetriol (**1**). Compounds **4**–**7** are new and have not been described previously.

RESULTS AND DISCUSSION

Repeated column chromatography of the ethyl acetate-soluble portions of the ethanol extract of *C. spicata* yielded **1** and **2**.

The spectroscopic data of **1** led to the structure 3,7-dimethyl-oct-1-ene-3,6,7-triol, a compound first isolated by Williams *et al.* [3] from *Vitis vinifera* var. Muscat Gordo Blanco. The ^{1}H NMR spectral data (60 MHz) published by Williams *et al.* were not given for the natural product but for the diastereomeric mixture of triols formed from diastereomeric 6,7-epoxy-linalooyl-acetate by acid catalysis [3]. Compound **1** isolated from *C. spicata* gave rise to a double set of signals in the ^{1}H NMR (500 MHz) consistent with a 10:1 mixture of two isomeric forms of 3,7-dimethyl-1-octene-3,6,7-triol. The spectroscopic data (Table 1) for the main isomer are given for the first time.

Based on the spectroscopic data (^{1}H, ^{13}C NMR, IR and FAB-MS), **2** is 3,7-dimethyl-octa-1,6-diene-3-ol-3-*O*-β-D-glucopyranoside (linalool-*O*-β-glucoside) (**2**) [4].

Chromatographic purification of the *n*-butanol-soluble portions of *C. spicata* yielded five monoterpeneglycosides (**3**, **4**/**5**, **6** and **7**). In accordance with the spectroscopic data, **3** was identified as 9-hydroxylinaloyl glucoside, a compound first described by Usmanghani [4].

The ^{1}H NMR spectrum of **4**/**5** indicated a mixture of two monoterpeneglycosides. Because of the small amount (1.1 mg) isolated, the mixture could not be separated further. Integration of the ^{1}H NMR spectrum established a 2:1 relationship between **4** and **5**. In addition to the H–H COSY, the different intensities of the signals permitted a clear assignment of the signals without further separation of the compounds. From the ^{1}H NMR spectrum a $^4C_1$ (D) conformation of the β-D-glucopyranosyl residues was indicated by the coupling constants ($J_{1',2'} \approx 8$ Hz, $J_{2',3'} \approx J_{3',4'} \approx J_{4',5'} \approx 9$ Hz). Based on the remaining ^{1}H NMR resonance signals and in accordance with the H–H COSY and the ROESY experiment, the aglycone of **4** and **5** had to be 3,7-dimethyl-octa-1,5-diene-3,7-ol (**4a**) and 3,7-dimethyl-octa-1-7-diene-3,6-ol (**5a**), respectively. The aglycones were synthesized according to the literature [5], starting from (±)-linalool, by ene-reaction and subsequent reduction with triphenylphosphine. The ^{1}H NMR spectrum of the products (**4a** and **5a**), measured in methanol-d_4, showed the same splitting patterns and roughly the same resonance frequencies as the resonances as the aglycone moieties of the glycosidic mixture. The position of the glycosidic linkage

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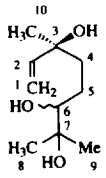

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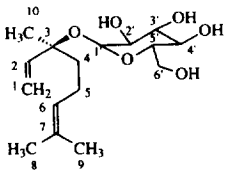

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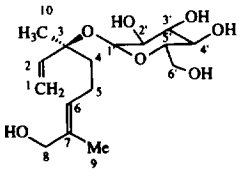

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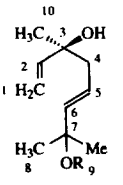
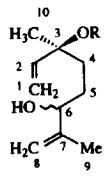


4 R = β-D-gluc

4a R = H



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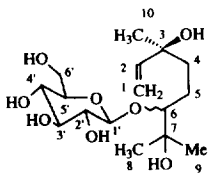
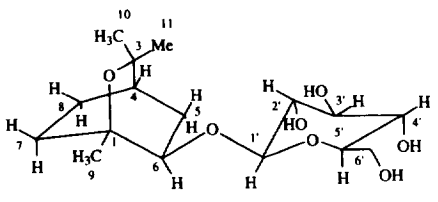
5a R = H



6



7

Table 1. ^{13}C and ^{1}H NMR spectral data of compound **1** (300/75 MHz, $CDCl_3$)

C/H	δ_C	δ_H a	δ_H b
1	111.9	5.07 *dd*	5.29 *dd*
2	145.2	5.93 *dd*	
3[a]	73.2	—	
4	39.0	1.79 *ddd*	1.66 *ddd*
5	26.0	1.59 *dddd*	1.40 *dddd*
6	78.7	3.38 *dd*	
7[a]	73.3	—	
8[b]	23.4	1.21 *s*	
9[a]	26.7	1.15 *s*	
10	28.1	1.31 *s*	

J (Hz): $J_{1a,1b} = 1.5$; $J_{1a,2} = 10.7$; $J_{1b,2} = 17.7$; $J_{4a,4b} = 15$; $J_{4a,5b} = 6.0$; $J_{4a,5a} = 8.8$; $J_{4b,5a} = 6.75$; $J_{5a,5b} = 15$; $J_{5a,6} = 2.25$; $J_{5b,6} = 10.5$.

[a,b]May be exchangeable.

was determined by using NOE difference spectroscopy. Upon irradiation of the anomeric protons of **4** and **5**, a NOE enhancement was observed for H-8 (Me) and H-9 (Me) of **4** as well as for H-2 (CH) and H-3 (Me) of **5**. According to this experiment and in agreement with the ROESY experiment, the structures of the two glycosides are 3,7-dimethyl-octa-1,5-diene-3,7-ol-7-*O*-β-D-glucopyranoside (**4**) and 3,7-dimethyl-octa-1,7-diene-3,6-ol-3-*O*-β-D-glucopyranoside (**5**). The ^{1}H and ^{13}C NMR spectra of **6** again revealed the presence of a β-D-glucopyranosyl residue (Table 2). Comparison of the spectral data of **6** ($^{1}H/^{13}C$ NMR, 1D and 2D techniques) with those of **1** pointed to the existence of a 6-*O*-β-D-glucopyranoside of 3,7-dimethyl-oct-1-ene-3,6,7-triol (**1**). The ^{1}H and ^{13}C NMR data of **6**, due to the aglycone, were in good accordance with those of **1**, except for the carbon resonance of C-6 which was displaced by +12 ppm indicating the glycosylation of the secondary alcoholic function [6, 7]. Furthermore, the deshielding of C-1′ (δ105) ruled out a tertiary alcoholic β-D-glucopyranoside. Hydrolysis of **6** with cellulase yielded a product whose ^{1}H NMR data were identical with those of **1**. Based on these findings, the overall structure was deduced to be 3,7-dimethyl-oct-1-en-3,6,7-triol-6-*O*-β-D-glucopyranoside (**6**).

The FAB-mass spectrum of **7** showed a molecular ion peak at *m/z* 333 $[M + H]^+$. In accordance with the ^{1}H

Table 2. ^{1}H and ^{13}C NMR spectral data of compound **6** (300/75 MHz, CD_3OD)

H/C	δ_H	δ_C
1a	5.19 *dd*	
		112.0
1b	5.01 *dd*	
2	5.89 *dd*	146.7
3	—	73.7[a]
	2.04 *m*	
4		39.5
	1.55 *m*	
	1.65 *m*	
5		27.3
	1.47 *m*	
6	3.46 *m*	90.4
7	—	74.0[a]
8	1.13 *s*[a]	26.6[b]
9	1.14 *s*[a]	24.5[b]
10	1.25 *s*	28.3
1′	4.34 *d*	105.1
2′	3.23 *dd*	75.4
3′	3.31 *m*	78.0
4′	3.36 *m*	71.5
5′	3.28 *m*	78.0
6a′	3.64 *dd*	
		62.5
6b′	3.85 *dd*	

J (Hz): $J_{1a,1b} = 1.5$, $J_{1a,2} = 10$, $J_{1b,2} = 16$; $J_{1',2'} = 7$, $J_{2',3'} = 8.5$, $J_{6a',6b'} = 10.5$, $J_{6a',5'} = 5$, $J_{6b',5'} = 1.8$.

[a,b]May be exchangeable.

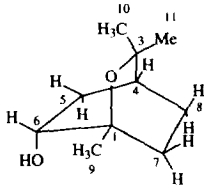
and ^{13}C NMR data (Table 4), the molecular formula was estimated to be $C_{16}H_{28}O_7$. Based on this evidence, three double bond equivalents were to be expected. Typical resonances of β-D-glucose in the NMR spectra as well as a lack of ^{13}C resonances in the olefinic region pointed to a bicyclic monoterpene glucoside. The spectroscopic data of the aglycone was consistent with a 1,8-cineole derivative, oxygenated at C-6. C-6 oxidation products of 1,8-cineole result in two pairs of enantiomeres (**7a**/**b** and **7c**/**d**) having an *endo* and *exo* orientation of H-6, respectively. The conformation at C-6 was determined by using NOE difference spectroscopy. Thus upon irradiation of H-6 an NOE enhancement was observed at H-7_{endo} and H-5_{endo}. According to this experiment and confirmed by the coupling constants (Table 4), H-6 was assigned to be *endo*. On the basis of this finding **7** must be the β-D-glucoside of **7c** or **d** having a 6*S* or 6*R* configuration. The β-D-glucosides of **7a** and **b** are known [8] and their configuration was derived on the basis of spectroscopic data. According to the literature [6,7], the chirality of glycosylated oxygen-bearing carbons in cyclic secondary alcohol β-D-glucosides can be determined by analysis of their ^{13}C NMR data. The chemical shifts of C-2 (δ80.2) and C-1′ (δ106.6) of the β-D-glucoside of **7b** were observed at lower field than those of **7a** [C-2, (δ76.3); C-1′ (δ102.3)], thus demonstrating a 6*S* configuration of the β-D-glucoside of **7b** and 6*R* configuration of the β-D-glucoside of **7a**, respectively. Compared to these shifts, **7** showed resonances for C-2 at δ74.3 and C-1′ at δ100.9 indicating a 6*R* configuration. Thus the structure of **7** is (1*S*,4*R*,6*R*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-*O*-β-glucopyranoside[(1*S*,2*R*,4*R*)-1,8-epoxy-*p*-menthan-2yl-*O*-β-D-glucopyranoside.

EXPERIMENTAL

^1H NMR: 300 and 500 MHz; ^{13}C NMR: 75 and 125 MHz; FAB-MS: thioglycerin as matrix; GPC: Sephadex LH 20; CC and TLC: silica gel, Si 60 (Lobar B, 40–63 μm, length: 310 mm, diameter: 25 mm, Merck), Rp-18 (Lobar C, 40–63 μm, length: 440 mm, diameter: 37 mm and Lobar A, 40–63 μm, length: 240 mm, diameter: 10 mm, Merck).

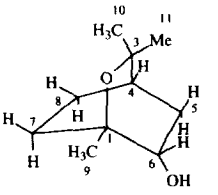
Plant material and isolation. Aerial parts of *Cunila spicata* were collected in December 1989 in Guaiba (Southern Brazil). A voucher specimen (herbarium no. 4/90-1): is deposited at the Pharmazeutisches Institut der Universität Bonn. Dried leaves of *C. spicata* (330 g) were extracted as previously described [2]. The EtOAc and the *n*-BuOH-soluble portions (1.5 and 2.8 g) were each separated into 5 fractions by gel permeation chromatography [GPC] (Sephadex LH-20) using MeOH–$CHCl_3$ (1:1) as eluant. The 2nd fraction from the EtOAc sepn was subjected to silica gel CC with $CHCl_3$–MeOH (6:1) as eluant and purified by LC on silica gel 60 (Lobar B, 40–63 μm, Merck) respectively, yielding 5 mg 3,7-dimethyl-1-octene-3,6,7-triol (**1**) and 15 mg linalool-*O*-β-glucoside (**2**). 3,7-dimethyl-1-octene-3,6,7-triol (**1**). Oil. TLC: $CHCl_3$–MeOH (6:1), R_f 0.36) $[\alpha]_D^{25}$ + 19° ($CHCl_3$; *c* 0.42; mixture of the diastereomeres, relation: 10:1); MS-data identical with lit. [3]; IR ν^{CHCl_3} cm^{-1}: 3680, 3600, 3400, 3090, 2940, 2880, 1645, 1460, 1380, 1170, 1090, 1000, 940; ^1H and ^{13}C NMR: Table 1.

Linalool-O-β-glucoside (**2**). Oil. TLC: $CHCl_3$-MeOH-H_2O (83:16:1; R_f 0.35). Spectral data identical with ref. [4]. The 4th fraction from the *n*-BuOH separation was subjected to RP_{18} (Lobar C) with H_2O-MeOH (4:1) as the eluent and purified by LC on RP_{18} (Lobar A) to yield



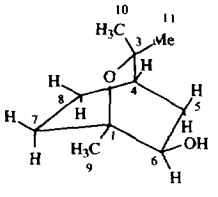
7a

(1R,4S,6R)



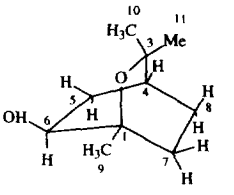
7b

(1S,4R,6S)



7c

(1S,4R,6R)



7d

(1R,4S,6S)

Table 3. ^1H NMR spectral data of the mixture of compounds **4** and **5** (500 MHz, CD_3OD)

H	4	5
1a	5.03 *dd*	5.13 *dd*
1b	5.19 *dd*	5.18 *dd*
2	5.93 *dd*	6.05 *dd*
4	2.28 *m*	
		1.7–1.5 *m*
5	5.67 m[a]	
6	5.67 *m*[a]	3.98 *m*
8a	1.30 *s*[b]	4.79 *br s*
8b		4.95 *br s*
9	1.33 *s*[b]	1.70 *s*
10	1.22 *s*[b]	1.32 *s*
1′	4.33 *d*	4.32 *d*
2′	3.11 *dd*	3.12 *dd*
3′	3.30 *m*	3.30 *m*
4′	3.25 *m*	3.26 *m*
5′	3.16 *m*	3.15 *m*
6a′	3.61 *dd*	3.62 *dd*
6b′	3.79 *dd*	3.79 *dd*

[a,b]May be exchangeable.

Table 4. ^1H and ^{13}C NMR spectral data of compound 7 (500/125 MHz)

H/C	δ_H	δ_C (CD$_3$OD)	δ_C (Pyridine-d_5)
1	—	73.9	71.0
3	—	75.7	73.1
4	1.54 *dddd*	34.8	33.3
5 *endo*	1.91 *ddd*		
		31.0	31.3
5 *exo*	2.10 *dddd*		
6	3.89 *dd*	75.0	74.3
7 *endo*	1.51 *ddd*		
		31.7	30.4
7 *exo*	1.74 *ddd*		
8 *endo*	1.40 *dddd*		
		22.7	21.9
8 *exo*	2.01 *ddddd*		
9	1.10 *s*	23.6	23.5
10	1.25 *s*	28.4	28.2
11	1.30 *s*	29.3	28.9
1′	4.37 *d*	100.6	100.9
2′	3.18 *dd*	74.9	74.4
3′	3.36 *dd*	78.1	78.4
4′	3.24	72.0	71.6
5′	3.23	78.1	78.3
6a′	3.63 *dd*		
		63.0	62.7
6b′	3.86 *dd*		

J (Hz): $J_{4,5en} = 3$, $J_{4,5ex} = 3.3$, $J_{4,8en} = 2.0$, $J_{4,8ex} = 3.1$, $J_{5en,5ex} = 15$, $J_{5en,6} = 9.8$, $J_{5ex,6} = 3.3$, $J_{5ex,8ex} = 3.3$, $J_{7en,ex} = 14$, $J_{7en,8en} = 11.5$, $J_{7en,8ex} = 2.6$, $J_{7ex,8en} = 6.1$, $J_{7ex,8ex} = 11$, $J_{8en,8ex} = 12.5$.

five monoterpene glucosides: **3** (1.5 mg), a mixture of **4** and **5** (1.1 mg), **6** (1.3 mg) and **7** (12 mg).

3,7-*Dimethyl-octa*-1,6-*diene*-3,8-*diol*3-O-β-D-*glucopyranoside* (**3**). Oil. TLC: $CHCl_3$–MeOH–H_2O (83:16:1; R_f 0.17). Spectral data identical with the ref. [4].

Mixture of 3,7-*dimethyl-octa*-1,5-*diene*-3,7-*diol*-7,O-β-D-*glucopyranoside* (**4**) *and* 3,7-*dimethyl-octa*-1,7-*diene*-3,6-*diol*-6-O-β-D-*glucopyranoside* (**5**). Oil. TLC: $CHCl_3$-MeOH-H_2O (83:16:1; R_f 0.16). ^1H NMR data: Table 3.

Synthesis. The aglycones **4a** and **5a** were prepared by photosensitized oxidation of linalool by a method described in ref. [5].

3,7-*Dimethyl-octa* 1,7-*diene*-3,6-*diol* (**4a**). Oil. TLC: hexane-EtOAc (3:1, R_f 0.16). ^1H NMR (300 MHz, CD_3OD): δ1.22 (3H, *s*, Me-10), 1.26 (6H, *s*, Me-8 and Me-9). 2.22 (2H, *m*, H-4), 5.01 (1H, *dd*, J = 10.8, 1.7 Hz, H-1a), 5.18 (1H, *dd*, J = 15.8, 1.7 Hz, H-1b), 5.62 (2H, *m*, H-5 and H-6), 5.92 (1H, *dd*, J = 15.8, 10.8 Hz, H-2); ^{13}C NMR (75 MHz, CD_3OD): δ 112.0 (C-1), 146.1 (C-2), 73.7 (C-3), 46.3 (C-4), 123.1 (C-5), 142.4 (C-6), 71.1 (C-7), 29.9 (C-8), 29.9 (C-9), 27.1 (C-10).

3,7-*Dimethyl-octa*-1,5-*diene*-3,7-*diol* (**5a**). Oil. TLC: hexane-EtOAc (3:2, R_f 0.23). ^1H NMR (300 MHz, CD_3OD): δ1.23 (3H, *s*, Me-10), 1.39-1.62 (4H, *m*, H-4 and H-5), 1.70 (3H, *t*, J = 0.5 Hz, H-9), 3.95 (1H, *t*, J = 6.5 Hz, H-6), 4.80 (1H, *quin*, J = 1.2 Hz, H-8a), 4.90 (1H, *quin*, J = 1 Hz, H-8b), 5.01 (1H, *dd*, J = 10.8, 1.7 Hz, H-1a), 5.18 (1H, *dd*, J = 17.7, 1.7 Hz, H-1b), 5.88 (1H, *dd*, J = 17.7, 10.8 Hz, H-2); ^{13}C NMR (75 MHz, CD_3OD): δ111.5 (C-1), 146.1 (C-2), 73.5 (C-3), 39.6 (C-4), 30.3 (C-5), 77.0 (C-6), 148.4 (C-7), 112.2 (C-8), 17.9 (C-9), 28.0 (C-10).

3,7-*Dimethyl*-1-*octene*-3,6,7-*triol*-6-O-β-D-*glucopyranoside* (**6**). Oil. TLC: $CHCl_3$–MeOH–H_2O (83:16:1; R_f 0.15). ^1H NMR and ^{13}C NMR: Table 3.

Enzymatic hydrolysis. Compound **6** (10 mg) and 40 mg cellulase (Merck) were dissolved in H_2O. The mixture was kept at 37° for 24 hr and then it was extracted with $CHCl_3$. The $CHCl_3$ layers were dried over Na_2SO_4 and evapd. The recovered aglycone of **6** (0.8 mg) and **1** showed identical NMR data.

(1S,4R,6R)-1,3,3-*Trimethyl*-2-*oseabicyclo* [2.2.2] *octan*-6-O-β-*glucopyranoside* (**7**). Oil. TLC: $CHCl_3$–MeOH–H_2O (83:16:1; R_f 0.24). FAB-MS *m/z*: 333 $[M + H]^+$. $[\alpha]_D^{20}$ − 53° (MeOH; *c* 0.4). ^1H and ^{13}C NMR: Table 4.

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REFERENCES

1. Pio Correa, M. (1984) *Dicionario das plantas uteis do Brasil e das exoticas cultivadas*, Vol. II. 205. Ministerio da Agricultura Instituto Brasileiro de desenvolvimento florestal.
2. Manns, D. and Hartmann, R. (1994) *Planta Med.* **60**, 467.
3. Williams, P. J., Strauss, C. R. and Wilson, B. (1979) *Phytochemistry* **19**, 1137.
4. Uchiyama, T., Miyase, T., Ueno, A., and Usmanghani, K. (1989) *Phytochemistry* **28**, 3369.
5. Matsuura, T. and Butsugan, Y. (1968) *Nippon Kagaku Zasshi* **89**, 513.
6. Kasai, R., Suzuo, M., Asakawa, J. and Tanaka, O. (1977) *Tetrahedron Letters* 175.
7. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y. (1977) *Tetrahedron Letters* 179.
8. Orihara, Y. and Furuya, T. (1994) *Phytochemistry* **35**, 641.