Phytochemistry, 1980, Vol. 19, pp. 2765 2766. Pergamon Press Ltd. Printed in England. 0031-9422/80/1201-2765 $02.00/0

SESQUITERPENE LACTONES OF *PODANTHUS MITIQUI*

MARITZA HOENEISEN,* MARIO SICVA* and FERDINAND BOHLMANN†

* Laboratory of Chemistry of Natural Products, Departmento de Botánica, Instituto Central de Biologia, Universidad de Concepción, Chile; † Institut für Organische Chemie der Technischen Universitat Berlin, Strasse des 17, Juni 135, D-1000 Berlin 12, W. Germany

(*Revised received* 28 *April* 1980)

Key Word Index *Podanthus mitiqui*; Compositae; sesquiterpene lactones; germacranolides; eudesmanolide; ovatifolin; deacetylovatifolin; 1 β-hydroxy-8β-angeloyloxyeudesmane-4(15),11(13)-diene-6α,12-olide; cytotoxic activity.

Abstract—Ovatifolin and two new sesquiterpene lactones, deacetylovatifolin and arturin (1β-hydroxy-8β-angeloyloxy-eudesmane-4(15),11(13)-diene-6α,12-olide, have been isolated from stems and leaves of *Podanthus mitiqui*. Two of these compounds showed cytotoxic activity.

INTRODUCTION

In connection with a screening programme of the Chilean flora for antitumor compounds [1], we wish to report the isolation of two new sesquiterpene lactones with cytotoxic activity [2–4].

Fractionation of the ethanolic extract of stems and leaves of *Podanthus mitiqui* Lindl., collected in October 1975 near Los Molles, Aconcagua, Chile, gave an aqueous methanolic extract with biological activity [2]. The methanolic extract was further purified by column chromatography on Si gel to yield three main compounds. The first compound, deacetylovatifolin (**1**) (found for $C_{15}H_{20}O_4$ M^+, m/e = 264, calculated 264.31) gave a typical ^{1}H NMR spectrum for a germacronolide skeleton with 8β,14-dihydroxy groups (Table 1). An IR absorption at 3400 cm^{-1} demonstrated the presence of the hydroxyl groups and absorptions at 1740 and 1660 cm^{-1} supported the presence of an α-methylene γ-lactone. The spectroscopic data and physical constants of this compound were very similar to those of budlein B but the rotation values were very different [5].

Table 1. ^{1}H NMR spectral data for compounds **1** and **2** (270 MHz, TMS as internal standard, $CDCl_3$)

	1	**2**
1-H	5.06 *dd(br.)*	5.14 *dd (br.)*
2,3-H	2.35 2.1 *m*	2.35–2.1 *m*
5-H	4.86 *d(br.)*	4.85 *d(br.)*
6β-H	5.12 *dd*	5.20 *dd*
7α-H	2.78 *m*	2.76 *m*
8α-H	4.49 *d(br.)*	4.59 *d(br.)*
9α-H	2.89 *dd(br.)*	2.94 *dd(br.)*
9β-H	2.40 *d(br.)*	2.20 *d(br.)*
13-H	6.36 *d*	6.36 *d*
13'-H	5.61 *d*	5.58 *d*
14-H	4.14 *d(br.)*	4.80 *d(br.)*
14'-H	3.83 *d(br.)*	4.58 *d(br.)*
15-H	1.68 *s*	1.63 *s*

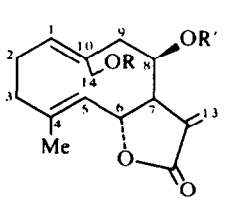
J(Hz): 1,2 = 11; 1,2' = 5; 5,6 = 6,7 = 10; 7,13 = 3.5; 7,13' = 3; 8,9 = 5; 14, 14' = 13.

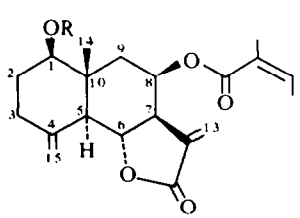
Further confirmation was obtained on treatment of compound **1** with acetic anhydride in pyridine. Two acetates were obtained, one identical to ovatifolin (**2**) and the other to ovatifolin acetate (**3**) [2, 3]. The spectral data suggested that the structure of the parent compound was **1**.

The second and most abundant compound ($C_{17}H_{22}O_5$, M^+ = 306) was found to be ovatifolin (**2**). ^{1}H NMR data (Table 1) as well as IR, mp and mmp against an authentic specimen confirmed that **2** was ovatifolin. This structure was further proved by X-ray diffraction [3].

The third compound, arturin (**4**) (found for $C_{20}H_{26}O_5$ M^+, m/e = 346.178, calculated 346.41) gave a typical ^{1}H NMR spectrum for a eudesmanolide skeleton with 1β-hydroxyl and 8β-angeloyloxy groups (Table 2). The spectrum showed a characteristic pair of doublets for H-13a,b.

An IR absorption band at 3460 cm^{-1} proved the presence of the hydroxyl group and bands at 1760 and 1650 cm^{-1} supported the presence of an α-methylene γ-lactone. The ^{1}H NMR spectrum showed signals at δ 5.04 and 4.96 typical for the methylene group at C-4. The data





1 R = R' = H
2 R = Ac; R' = H
3 R = R' = Ac

4 R = H
5 R = Ac

Table 2. ^{1}H NMR spectral data for compound **4** (270 MHz, TMS as internal standard, $CDCl_3$).

1α-H	3.54 *dd*
2α-H	1.93* *m*
2β-H	1.64* *m*
3α-H	2.15 *ddd*
3β-H	2.37 *ddd*
5α-H	2.28 *d(br.)*
6β-H	4.52 *dd*
7α-H	2.87 *dddd*
8α-H	5.84 *ddd*
9β-H	2.41 *dd*
9α-H	1.64* *m*
13-H	6.18 *d*
13′-H	5.50 *d*
14-H	0.98 *s*
15-H	5.04 *s(br.)*
15′-H	4.96 *s(br.)*
3′-H	6.11 *qq*
4′-H	1.93 *dq*
5′-H	1.86 *dq*
OAc	2.07 *s*

* Overlapped signals. All assignments are established by double resonance experiences.

J (Hz) = 1α, 2α = 4,5; 1α,2β = 11; 2β, 3α = 13; 2β, 3β = 6; 2α, 3α = 6; 2α, 3β = 1.5; 3α, 3β = 13; 5α, 6β = 11; 6β, 7α = 11; 7α, 8α = 2.5; 7α, 13 = 3.5; 7α, 13′ = 3; 8α, 9α = 8α; 9β = 2.5; 9α, 9β = 15; 3′,4′ = 7; 3′,5′ = 4′,5′ = 1.5.

showed that this compound was related to reynosin [6]. Therefore, on the basis of these data, arturin was assigned structure **4**.

Ovatifolin and arturin showed cytotoxic activity against human epidermoid carcinoma of the nasopharinx (KB). The ED_{50} values were 2.2 × 10(0) and 4.2 × 10(0), respectively.

EXPERIMENTAL

Mps are uncorr. Si gel GF 254 (Merck) was used for TLC plates.

Dried powdered stems and leaves were extracted with EtOH at 50° with stirring. The ethanolic extract was fractionated and the $CHCl_3$-soluble fraction was further treated with petrol and 10% aq. MeOH. Activity was found in the aq. methanolic fraction. Chromatographic separation of 220 g of the aq. MeOH extract on a Si gel column afforded the following compounds:

Deacetylovatifolin (**1**). This compound was eluted with petrol (bp 60–80°)–EtOAc (60:40) to yield after crystallization the pure compound (30 mg), mp 165°; $[\alpha]_D^{20}$ −259° (MeOH, *c* 0.92); UV λ_{max}^{MeOH} nm: 210 (ε 8052); IR ν_{max}^{Nujol} cm^{-1}: 3400, 1740, 1660, 1300, 1240, 1155, 1040, 950, 907, 860, 840, 740 and 690; MS *m/e*: M$^+$ 264 (calc. for $C_{15}H_{20}O_4$ 264.31), 257, 246, 228, 210, 165, 136, 120, 105, 91, 77. Treatment of this compound with Ac_2O in pyridine at room temp. gave a mixture of ovatifolin (**2**) and ovatifolin acetate (**3**). The latter was the main compound and both were identical with authentic samples.

Ovatifolin (**2**). Upon crystallization of the second compound (from EtOH) pure ovatifolin was obtained (400 mg), mp 135°, $[\alpha]_D^{25}$ −63.3 ($CHCl_3$, *c* 1.36), UV λ_{max}^{MeOH} nm: 211 (ε 23 256), IR ν_{max}^{Nujol} cm^{-1}: 3450, 2980, 1750, 1660, 1235 and 725; MS *m/e*: M$^+$ 306 (calc. for $C_{17}H_{22}O_5$ 306.35), 264, 246, 228, 217, 213, 166, 121, 107, 91, 43. This compound was identical to authentic ovatifolin. The structure was further confirmed by X-ray diffraction (**2**).

Ovatifolin acetate (**3**) was obtained by treatment of ovatifolin (**2**) with Ac_2O in pyridine at room temp. The acetate had mp 152°; $[\alpha]_D^{25}$ −15° ($CHCl_3$, *c* 1.0); UV λ_{max}^{MeOH} nm: 212 (ε 10 000), IR ν_{max}^{Nujol} cm^{-1}: 2970, 1770, 1740, 1248, 1258, 1040 and 970.

Arturin (*1β-hydroxy-8β-angeloyloxyeudesmane-4(15),11(13)-diene-6,12-olide*, **4**). Further elution of the column gave arturin (**4**) as an oil, UV λ_{max}^{MeOH} nm: 210 (ε 4692); IR ν_{max}^{Nujol} cm^{-1}: 3460, 2960, 2860, 1760, 1720, 1650, 1230, 1150, 1040, 1020, 990, 960 and 750; MS *m/e*: M$^+$ 346 (calc. for $C_{20}H_{26}O_5$ 346.178). Acetylation of arturin (**4**) with Ac_2O in pyridine gave arturin acetate (**5**), as an oil. UV λ_{max}^{MeOH} nm: 213 (ε 3768), IR ν_{max}^{Nujol} cm^{-1}: 2940, 1780, 1730, 1665, 1460, 1370, 1250, 1150, 1080, 1030, 960, 895 and 750.

Acknowledgements—We wish to thank Professor C. Marticorena for the botanical identification, Dr. Alonso Romo de Vivar for an authentic sample of budlein B, Mr. Z. Rozas for the technical assistance and Mrs. G. Torres for the preparation of the manuscript. We would like to acknowledge the financial support from the Organization of The American States, CONICYT (Chile), Vicerrectoría de Investigación, Universidad de Concepción and the National Cancer Institute (U.S.A.) for the testing facilities.

REFERENCES

1. Bhakuni, D. S., Bittner, M., Marticorena, C., Silva, M., Weldt, E. and Hoeneisen, M. (1976) *Lloydia* **39**, 4, 225.
2. Hoeneisen, M., Silva, M. and Watson, W. H. (1979) *Rev. Latinoam. Quim.* (in press).
3. Gopalakrishna, E. M., Watson, W. H., Hoeneisen, M. and Silva, M. (1977) *J. Cryst. Mol. Struct.* **7**, 49.
4. Anon. (1962) *Cancer Chemother. Rep.* **25**, 1.
5. de Vivar, A. R., Guerrero, C., Díaz, E., Bratoeff, E. A. and Jimenez, L. (1976) *Phytochemistry* **15**, 525.
6. Yoshioka, H., Renold, W., Fisher, N. H., Higo, A. and Mabry, T. J., (1970) *Phytochemistry* **9**, 823.