Pergamon

PII: S0031-9422(98)00436-1

Phytochemistry Vol. 49, No. 5, pp. 1475–1477, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

AN ABIETANE FROM *TAXUS CUSPIDATA* CELL SUSPENSION CULTURES

KRISTINA SALCICCIOLI,† FRANK DICOSMO†,‡,§* and WILLIAM F. REYNOLDS‖

†Department of Botany University of Toronto, 25 Wilcocks Ave, Toronto, Ont., Canada M5S 3B2, ‡Institute of Biomedical Engineering, University of Toronto, Toronto, Ont., Canada M5S 2A1, §Department of Surgery, University of Toronto, Toronto, Ont., Canada M5S 2A1 and ‖Department of Chemistry, University of Toronto 80 St. George St., Toronto, Ont., Canada M58 2A1

(*Received* 7 *November* 1997)

Key Word Index—*Taxus cuspidata*; cell culture; abietane; 3,20-epoxy-12-methoxy-8,11,13-abietatriene-3,7,11-triol.

Abstract—An abietane diterpenoid was isolated from an ethyl acetate extraction of *Taxus cuspidata* cell suspension media. The structural assignments were established by mass spectroscopy, and ^{1}H and ^{13}C NMR studies including COSY, HMQC and HMBC. This compound was determined to be 3,20-epoxy-12-methoxy-8,11,13-abietatriene-3,7,11-triol. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Taxus species, such as *T. baccata*, *T. brevifolia*, *T. canadensis* and others have attracted a great deal of attention as the sources for the anticancer agent paclitaxel, and related taxoid compounds in order to meet the growing demands for this natural product [1, 2]. Research has been directed toward semisynthesis [3, 4], total synthesis [5, 6], improving extraction techniques, and the use of cell cultures of various *Taxus* species as alternative sources of this drug [7, 8]. Research into the use of inducers and precursor feeding continues in this area [9, 10]. As part of our ongoing examination of the isolation of taxanes from cell cultures of *T. cuspidata*, an abietane-type diterpenoid (**1**), was isolated from the liquid broth of six-year-old suspension cultures. The abietane (**1**) is a relatively abundant metabolite that exhibits the characteristic abietane motif. The abietanes are widely distributed; abietic acid is a common constituent of tree resins [11], carnosic acid is found in *Rosmarinus officinalis* [12], and royleanone and related compounds occur in *Salvia lavandulaefolia* [13].

RESULTS AND DISCUSSION

Abietane (**1**) was isolated as fine white crystals in 0.088 mg/L yield (±0.0005 mg/L). UV analysis demonstrated λ_{max}^{MeOH}nm = 210, 225, 280. High resolution electron impact spectroscopy established the molecular formula to be $C_{21}H_{30}O_5$. The carbon skeleton was established on the basis of ^{1}H and ^{13}C NMR spectral data (Table 1). Figure 1 exhibits the characteristic abietane three fused ring structure, isopropyl group (carbons 15–17), and two methyl groups (C_{18}/C_{19}) substituted on C-4. This compound demonstrates a high degree of substitution having a methoxy group at C-12 (δ_c 61.8), two alcohol groups ($\delta_{c\text{-}3}$ 98.3, $\delta_{c\text{-}7}$ 68.8), a phenolic group ($\delta_{c\text{-}11}$ 140.4) and a bridging cyclic herni-ketal to C-3 ($\delta_{c\text{-}3}$ 98.3) from C-20 ($\delta_{c\text{-}20}$ 66.2). H-7 showed very small couplings to both C-6 protons, suggesting that the former is in a pseudoequatorial position. This is also supported by the results of the HMBC spectrum which showed strong 3-bond connectivities to H-7 to C-5, C-9 and C-14. This suggests that H-7 is in an anti orientation with respect to C-5 and C-9 and syn with respect to C-14, an observation only consistent with it being in a pseudoequatorial position. Construction of a molecular model suggests that the probable configuration/conformation has the C-7-OH on the α-face with ring B in half-chair form.

EXPERIMENTAL

Extraction

The B5C2 FLC2 *Taxus cuspidata* cell suspension cultures were obtained from existing stocks. The

*Author to whom correspondence should be sent.

Table 1. NMR spectral data interpretation for abietane*

Position	δ_C	δ_H	C-H 2 or 3 bond connectivities	^1H-^1H connectivities
1	29.9	1.49m, 1.38	3.90	1.86, 2.28, 3.38
2	29.1	2.28m		1.86, 1.49, 3.38
		1.86m		1.49, 2.28, 3.38
3	98.3		1.04, 1.15, 4.63,	
4	39.9		1.04, 1.15	
5	41.1	2.20m	1.04, 1.15, 3.90, 4.79	1.94, 1.80
6	27.8	1.94m		1.80, 4.79
		1.80m		2.20, 4.79
7	68.8	4.79m	1.94, 6.78	
8	132.1		6.78	
9	122.0		4.79, 6.08, 6.78	
10	41.6		1.86, 3.90, 4.63	
11	147.9		6.08	
12	144.4		3.74, 6.78	
13	140.4		1.22, 3.20	
14	119.2	6.78s	3.20, 4.79	
15	26.4	3.20m	1.22, 6.78	1.22
16	23.4	1.22d	1.22, 3.20	3.20
17	23.4	1.22d	1.22, 3.20	1.22
18	18.1	1.04s	1.15	
19	26.3	1.15s	1.04	
20	66.2	3.90d		4.63
		4.63d		3.90
OCH_3	61.8	3.74s		
C-11-OH		6.08s		

*NMR parameters are listed in the Section 3.

spent liquid broth was collected from the cell cultures during subculturing which occurred during the 20–22 days of the cellular growth cycle. The media were reduced to approximately one tenth of the original volume by drying on a rotovaporator. A total of ten litres of broth was used for each extraction. The concentrated broth (300 mL) was extracted with an equal amount of ethyl acetate four times. The organic extracts were collected and were dried completely then resuspended in 12 mL of ethyl acetate. Organic extracts were stored at −20°C.

Organic component separation and purification

Whatman Sep-pac plus silica cartridges were used for a preliminary separation of organic components. The columns have surface pH = 7, pore size 60 Å, and particle size of 125 mm (part number 20520 lot number P4137A3). All washes and organic extracts were applied using a 3 cm^3 syringe and positive pressure. The columns were prepared with a 1.5 mL × 2 wash of hexane. A 0.40 mL quantity of extract was then applied to the column. A 1.5 mL × 2 wash of hexane was applied. Washes of increasing polarity (1.5 mL × 2) were then applied (90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 50:50, 40:60, 20:80 all hexane:ethyl acetate respectively). A final wash with 100% ethyl acetate completed the column washes. Each eluant fraction was dried down in a Vortex evaporator at 35°C. Each fraction was resuspended in 150–200 mL HPLC grade methanol, filtered through 0.45 mm filters and subjected to HPLC analysis. HPLC separation was achieved using a C-18 phenyl bonded silica gel reverse phase analytical column. A linear gradient elution of methanol:water-acetonitrile 20:67:13 progressing to 20:27:53 v/v (volume/volume) over 50 min. was used A five min wash to reverse the gradient to initial conditions concluded each run. The flow rate was 1 mL per min, with injection volume of 40–20 mL. The elution of the organic components was monitored by photodiode array UV detection at 227 and 550 nm. Compound **1** had a retention time of 18.34 min. The compound was retained on ice between collections. The eluted fractions were dried, resuspended in 100 mL of HPLC grade methanol, and filtered through a 0.45 μm filter. All HPLC analyses were performed with a Hewlett-Packard 1090A high performance liquid chromatographic system. Purified samples were dried under N_2 for 3 h. The compound was stored under nitrogen in darkness at −20°C.

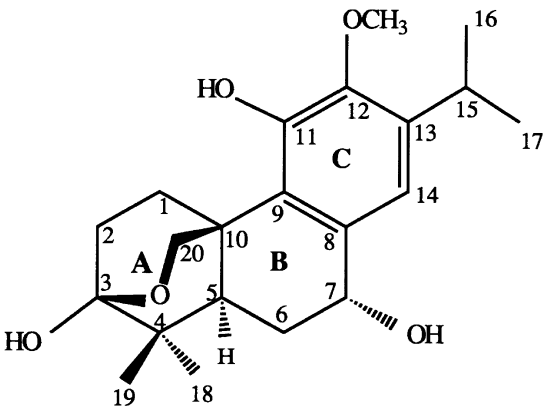

Fig. 1. 3,20-Epoxy-12-methoxy-8,11,13-abietatriene-3,7,11-triol from *T. cuspidata*.

NMR spectral parameters

^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC spectra were obtained on a Varian Unity 500 NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{13}C spectra were obtained with a 5 mm heteronuclear probe while ^{1}H and two-dimensional spectrum were obtained with a 5 mm inverse detection probe. The sample consisted of 500 μg of the compound dissolved in 1 mL of $CDCl_3$, containing 0.01% TMS to provide a reference signal. Sample temperature was maintained at 25°C during spectral acquisition. All spectra were obtained using standard Varian software and acquisition parameters. Measuring times were 30 min for the ^{1}H spectrum, 16 h for COSY and HMQC spectra, 24 h for the ^{13}C spectrum and 64 h for the HMBC spectrum.

Mass spectroscopy analysis

A 6.5 L extraction was used to obtain sufficient amounts of the component for mass spectral analysis. The procedure previously described was followed. The compound was purified, to recover 0.55 mg ($\pm$0.005 mg). The component's identity was confirmed by 1-D ^{1}H NMR. The Fison 70-250S double focusing mass spectrometer used for the analysis utilized a 8 keV accelerating voltage, with a source temperature of 250°C at a source pressure of 10^{-6} mbar. Both high and low resolution mass spectra were obtained. EIMS 70 eV m/z (rel. int.) chemical formula-344 (10.7) $[M–H_2O]^+$, 313.2 (42.7) $[M–H_2O–OCH_3]^+$, 281.2 (66.7) $[M–H_2O–H_2O–CH_3–HCOH]^+$, 271.2 (45.8) $[M–OH, –OCH_3, –CH(CH_3)_2]^+$, 270.2 (56.1) $[M–H_2O, –OCH_3, –CH(CH_3)_2]^+$, 229.2 (100) $[M–HCOH, –OH, H_2O, CH_2CC(CH_3)_2]^+$.

REFERENCES

1. Fett-Neto, A. G. and DiCosmo, F., *Planta Med.*, 1992, **58,** 464.
2. Kingston, D. G. I., Molinero, A. A. and Rimoldi, J. M., *Progr. Chem. Org. Nat. Prod.*, 1993, **61,** 1.
3. Holton, R. A., U.S. Patent No. 5 015 744, 1994.
4. Kingston, D. G. I., Chaudhary, A. G., Guntilaka, A. A. L. and Middleton, M. L., *Tetrahedr. Lett.*, 1994, **35,** 4486.
5. Nicolaou, K. C., Yang, Z., Liu, J. J., Ueno, H., Nantermet, P. J., Guy, R. K., Claiborne, C. F., Renaud, J., Couladouros, E. A., Paulvannan, K. and Sorensen, E. J., *Nature*, 1994, **367,** 630.
6. Holton, R. A., Sornoza, C., Kim, H. B., Liang, F., Biediger, R. J., Boatman, P. D., Shindo, M., Smith, C. C., Kim, S., Nadizadeh, H., Suzuki, Y., Tao, C., Vu, P., Tang, S., Zhang, P., Murthi, K. K., Gentile, L. N. and Liu, J. H., *J. Am. Chem. Soc.*, 1994, **116,** 1597.
7. Fett-Neto, A., DiCosmo, F., Reynolds, W. and Sakata, K., *Bio/Technology*, 1992, **10,** 1572.
8. Seki, M., Ohzora, C., Takeda, M. and Furusaki, S., *Biotechnol. Bioeng*, 1997, **58,** 214.
9. Fett-Neto, A. G., Melanson, S. J., Nicholson, S. A., Pennington, J. J. and DiCosmo, F., *Biotechnol. Bioeng.*, 1994, **44,** 967.
10. Yukimune, Y., Tabata, H., Higashi, Y. and Hara, Y., *Nat. Biotechnol.*, 1996, **14,** 1129.
11. Dockerell, B. and Hanson, J. R., *Phytochemistry*, 1978, **17,** 1119.
12. Gonzalez, A. G., Andres, L. S., Javier, G. L., Brito, I. and Rodriguez, M. L., *Phytochemistry*, 1991, **30,** 4067.
13. Michavilia, A., Fernandez-Gadea, F. and Rodriguez, B., *Phytochemistry*, 1986, **25,** 226.