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Isolation and Confirmation of the Proposed Cleistanthol Biogentic Link from *Croton insularis*

Lidia A. Maslovskaya,[†] Andrei I. Savchenko,[‡] Victoria A. Gordon,[§] Paul W. Reddell,[§] Carly J. Pierce,[†] Peter G. Parsons,[†] and Craig M. Williams*[,‡]

School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane 4072, Queensland, Australia, Queensland Institute of Medical Research, P.O. Royal Brisbane Hospital, Brisbane 4029, Queensland, Australia, and EcoBiotics Limited, P.O. Box 1, Yungaburra, 4884 Queensland, Australia

c.williams3@uq.edu.au

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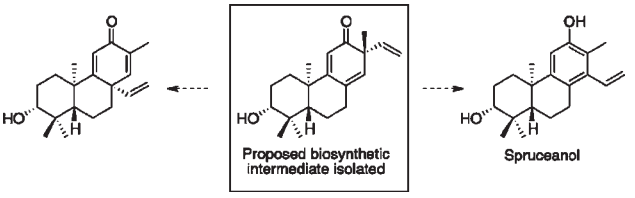
ABSTRACT



The proposed cleistanthol biosynthetic intermediate en route to spruceanol, and other related family members, was isolated for the first time from *Croton insularis*, confirming the Jacobs−Reynolds hypothesis. Anticancer evaluation of the new isolates and their aerial oxidation products is also reported.

In 2001, Jacobs and Reynolds isolated from *Jatropha divaricata* two pimarane-type diterpenes (**1** and **2**) of which **2** was a new skeletal type (Scheme 1).[1] Considering two known members of the rare cleistanthane series[2] of diterpenes [spruceanol (**3**)[3] and cleistanthol (**4**)][4] were isolated together with **1** and **2**,[5] Jacobs and Reynolds surmised that a close biogenetic link most likely exisited between the three sketal types (i.e., **1**−**3**).

This notion was further supported by the fact that pimarane derivatives have been converted by acid catalysis to the cleistanthane system previously.[6]

[†] University of Queensland.
[‡] Queensland Institute of Medical Research.
[§] EcoBiotics Limited.

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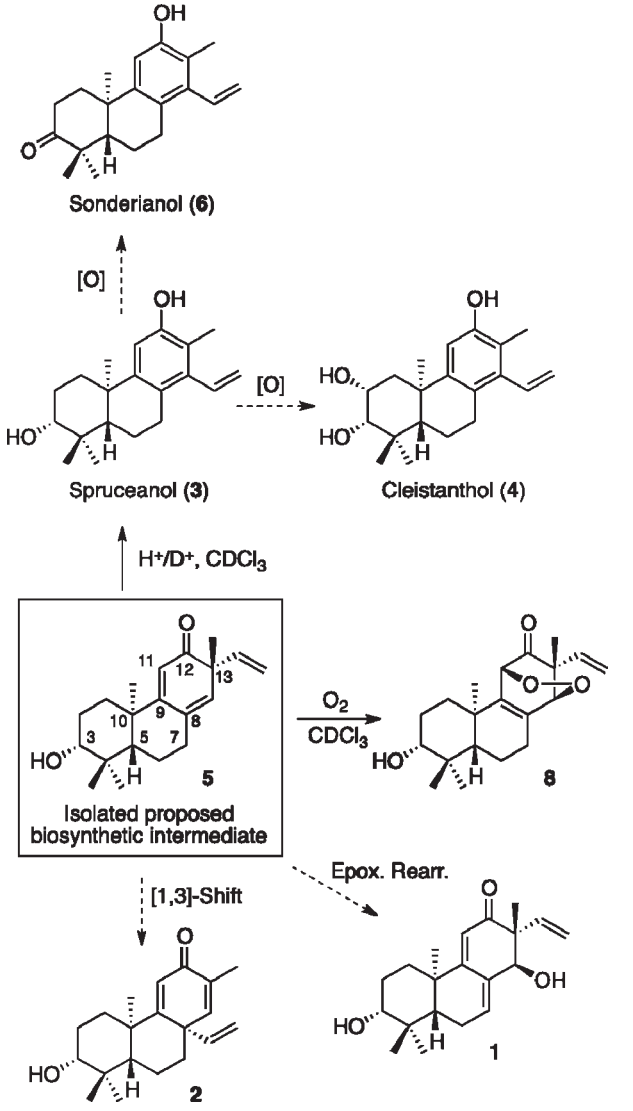
Scheme 1. Relationship between the Proposed Biogentic Link **5** (and or **7**) to Pimaranes (**1** and **2**) and the Cleistanthane Ditepenes (i.e., **3** and **6**). Conversion of **5** into Spruceanol **3**, Confirming the Biogentic Link, and the Peroxy Derivative **8**



With this knowledge in hand, the *ent*-3β-hydroxypimara-8(14),9,15-trien-12-one **5** was proposed as the most probable biosynthetic intermediate. Further justification for **5** was outlined with plausible biosynthetic routes to skeletons **1**−**3** (Scheme 1). For example, **5** could conceivably undergo aromatization via a 1,2-shift of the vinyl moiety contained in the C-ring to give spruceanol (**3**); epoxidation of the 8,14 double bond and subsequent rearrangement would lead to **1**, and either a [1,3]- or [2,3]-sigmatropic shift of the vinyl group from C13 to C8 arrives at **2** (Scheme 1).

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In the course of our ongoing search for natural products displaying anticancer activity,[7,8] we investigated *Croton insularis* (Baillon), a plant endemic to northeast Australian rain forests, driven by previous accounts of biological screening on this species.[9]

We report herein isolation of the proposed biogenetic link pimarane **5**, its corresponding keto derivative **7**, together with spruceanol (**3**) and sonderianol (**6**).[2b,10]

Table 1. ^{1}H and ^{13}C NMR Data for Compound **5** Recorded in $CDCl_3$ at 500 MHz

position	^{1}H, σ (ppm)	multiplicity	J, Hz	^{13}C, σ (ppm)
1a	1.54	1H (dd)	13.20, 3.91	35.8
1b	1.99	1H (dt)	13.20, 3.42	
2a	1.68−1.73	1H (m)		27.5
2b	1.73−1.78	1H (m)		
3	3.25	1H (dd)	11.74, 4.40	78.2
4				39.5
5	1.21	1H (dd)	8.31, 3.91	47.7
6a	1.64−1.68	1H (m)		19.1
6b	1.78−1.83	1H (m)		
7a	2.43−2.52	1H (m)		29.2
7b	2.74	1H (d)	16.14	
8				129.3
9				166.9
10				38.8
11	5.81	1H (s)		117.7
12				204.3
13				52.6
14	5.91	1H (s)		139.2
15	5.73	1H (dd)	17.12, 10.27	139.9
16a	5.00	1H (d)	10.27	114.1
16b	5.03	1H (d)	17.12	
17	1.26	3H (s)		23.4
18	1.02	3H (s)		27.9
19	0.88	3H (s)		15.4
20	1.14	3H (s)		23.0

HRESIMS analysis of **5** ($[M + Na]^+$, m/z 323.1982), in conjunction with a well deconvoluted ^{13}C NMR spectrum, established a molecular formula of $C_{20}H_{28}O_2$. The ^{1}H NMR (Table 1) spectrum displayed characteristic resonances for the vinyl moiety, two olefinic protons (δ_H 5.8 and 5.9), one oxygenated methine (δ_H 3.2), isolated axial ring protons (δ_H 2.7, 2.5 and 2.0), and four methyl groups. The ^{13}C NMR (Table 1) spectra revealed all 20 carbons, which in combination with DEPT measurements were found to be four methyl groups, five methylenes (one olefinic), five methines (three olefinic, one oxygenated, and one saturated), and six quaternary centers (two olefinic,

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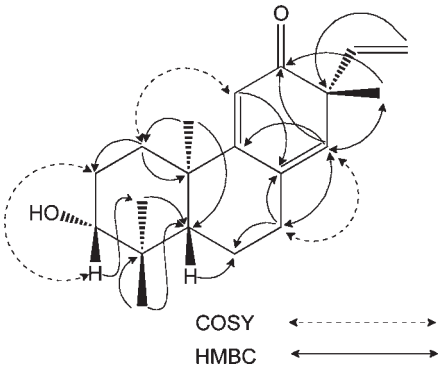


Figure 1. COSY and HMBC correlation diagram.

three saturated, and one carbonyl carbon). Key $^{1}H-^{1}H$-COSY, HSQC, and HMBC correlations (see Figure 1), in conjunction with the ACD/laboratories software[11] and reported ^{13}C chemical shifts for similar diterpenes,[12] established the position of the hydroxyl at C3. This in turn initiated the connectivity of the A ring and angular methyl, both of which instate C10 and C9. H14 (δ_H 5.9) and H11 (δ_H 5.8) ascribed the position of the ajacent carbonyl and quaternary olefinic carbons (C8 and C9). Subsequent correlations in the $^{1}H-^{1}H$-COSY, HSQC, and HMBC build the remaining C ring olefin and quaternary carbon (δ_C 52.6) containing the methyl (δ_C 23.4) and vinyl moiety. The B ring methylene at C7 (δ_C 29.2) and H5 (δ_H 1.2) pin points the second methylene carbon C6 (δ_C 19.1).

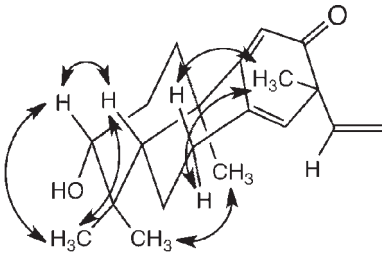
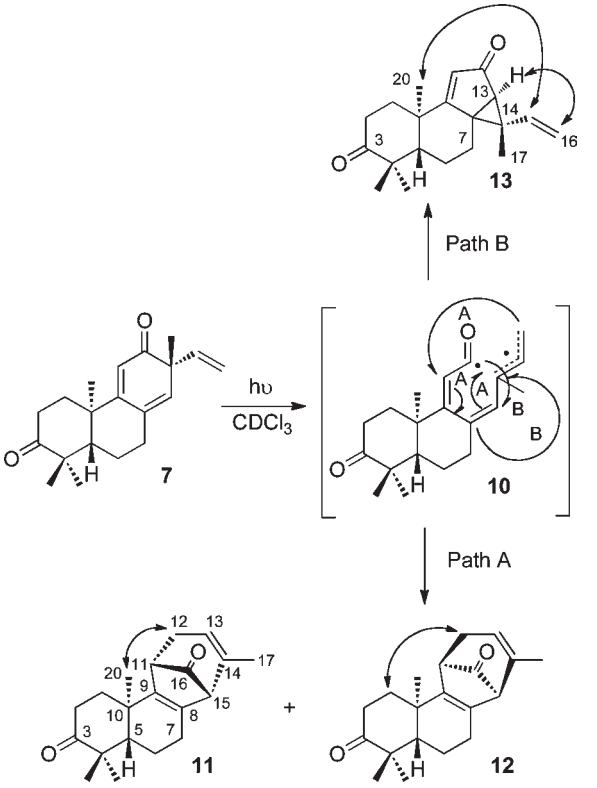


Figure 2. NOESY correlation diagram for compound **5**.

NOESY experiments (Figure 2) confirmed the 1,3-diaxial *syn* relationship of H3 and H5 and the *trans*-decalin system in compound **5**. Only weak correlations between the C13 methyl and H7α and H7*β* could be detected so the stereochemistry at this position is assigned based on that argued by Jacobs and Reynolds.[1]

With the isolation of **5** now established, chemical conversion was sought to provide definitive proof that **5** is indeed the stated biogentic link to diterpenes **1**−**3** (Scheme 1). When a solution of **5** in deuterated chloroform was allowed to slowly evaporate in air three products could be identified. An inseparable mixture of exo- and endoperoxides **8** and **9** (**9** not shown) along with the key product, spruceanol (**3**), confirmed beyound doubt the Jacobs−Reynolds hypothesis.

Scheme 2. Norrish Type 1 Photodegradation of Compound **7** Giving Derivatives **11**, **12** (via Path A) and **13** (via Path B)[a]



[a] Double-headed arrows indicate NOESY correlations.

The elucidation of the C3 keto **7** derivative was straightforward when compared to the spectra obtained for that of **5**. Exposure of **7** to ambient laboratory light in $CDCl_3$ unexpectedly afforded three Norrish type 1 photodegraded products via intermediate **10**. Bicyclic derivatives **11** and **12** (1:1 mixture) via pathway A and the cyclopropane **13** via pathway B (Scheme 2).

The elucidation of **11** and **12** was based on the fact that it is a decomposition product of **7**. The ^{13}C NMR chemical shifts of rings A and B atoms did not change drastically. However, according to ^{13}C NMR and HSQC correlations, **11** and **12** only have two double bonds with three quaternary carbons (i.e., C8, C9, C14), one CH sp^2 carbon (C13), and possess eight ring and double bond equivalents as seen in **7**. The key feature was two new sp^3 CH carbons C11 (46.6) and C15 (55.3), clearly indicating the formation of an additional ring instead of double bond. Correlations involving, but not between, H11, H15, and methyl group C17 in the $^{1}H-^{1}H$-COSY and the HMBC were key for building the additional unsaturated ring and showed the

(11) ACD/H and C NMR Predictor; ACD/Structure Elucidator, version 10.0, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2007; www.acdlabs.com.

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Table 2. Inhibition of Growth of Human Cell Lines in Culture

		ED_{50} (μg/mL)						
cell line	origin	**3**	**6**	**5**	**7**	**11**	**12**	**13**
NFF	normal fibroblasts	23	23	38	20	50	>100	70
HeLa	cervical carcinoma	3.4	13	16	2.7	25	20	53
HT 29	colon cancer	12	13	15	4.8	50	35	55
MCF-7	breast cancer	18	16	22	19	43	>100	60
MM96L	melanoma	5.1	2.8	7.4	0.6	28	45	28
K562	leukemia	13	17	23	0.47	60	>100	90

double-bond migration to positions 8 and 9. The elucidation of **13** began with the analysis of the HMBC correlation between the C20 methyl indicating the position of an sp^2 atom at position 9. Additional HMBC cross peaks involving, but not between, H11 and methyl C17 determined the position of the carbonyl on C12 adjacent the cyclopropane ring. The regiochemistry of derivatives **11**−**13** was obtained from NOESY spectra (see the Supporting Information), which shows specific correlation H12−H1 observed only for **12** and H12−H20 found only for **11**. Compound **13** has clear NOESY correlations H15−H20, H15−H13, and H16−H13 corresponding to the structure depicted (Scheme 2).

Biological evaluation of **3**, **5**−**7**, and **11**−**13** was performed against a panel of human tumor cell lines (Table 2). Clonogenic-type assays for comparing the growth of treated cells showed some inhibitory activity for **3**, **6**, and **5** in a normal cell type (NFF) and in a range of tumor cell lines (Table 2). The results from **7**, however, were notable because of a 5- to 20-fold potency increase in four of the five tumor cell lines compared with the other compounds and selectivity against tumor cells compared with normal fibroblasts.

The MM96L line is sensitive to killing by oxidants released during autoxidation of catechols,[13] but autoxidation does not adequately account for the potency of **7** because **5** oxidized in air to the endoperoxide **8** at a similar rate yet was less active. Although **1** and **2** have not undergone biological evaluation,[14] both spruceanol (**3**) and sonderianol (**6**) have previously been evaluated. Spruceanol (**3**) displayed marginal activity (ED_{50} 3.2 μg/mL) against the P-388 test system in vitro,[3] whereas sonderianol (**6**) displayed cytotoxicity against leukemia L1210 (and P388) cells in mice (100% growth inhibition at a concentration of 50 μg/mL) and liver cancer HEPAlclc7 cells in mice (IC_{50} 0.403 mmol/L).[15]

In conclusion, the Jacobs−Reynolds proposed biogenetic link, pimarane **5**, between pimarane and cleistanthane diterpenes has been isolated, elucidated, and chemically proven.

Acknowledgment. We thank EcoBiotics Ltd. and the University of Queensland for financial support. Dr. Lynette Lambert from the Centre of Advanced Imaging at the University of Queensland for assistance with NMR measurements.

Supporting Information Available. Experimental procedures and copies of ^1H, ^{13}C and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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