Sample Scientific Paper

Identification of Chemical Constituents in the Rhizomes of *Hedychium coronarium*

Milly L.C. Chang and Dr. Leticia Colmenares
Department of Natural Sciences, Windward Community College, 45-720 Keaahala Rd, Kaneohe, Hawaii, 96744

Personal Reflection

This was my first real laboratory research project, so I gained a plethora of knowledge about general laboratory basics and techniques. I learned about lab safety, lab tools, and various organic chemistry procedures like thin layer chromatography (TLC), high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy, and more.

Introduction

The Zingiberaceae plant *Hedychium coronarium*, white ginger lily, is widely cultivated in Southeast Asia, India, China, Japan, Brazil, and so on. The plant is commonly known as "awapuhike'oke'o" in Hawaiian, "tuqianghou" in Chinese, "mahahong" in Thai, and "dolan champa" in Hindi. The rhizomes of *H. coronarium* have been prescribed in traditional medicine for the treatment of sharp pain due to rheumatism, contusion inflammation, and headaches.

This research focused on the isolation of pure chemical constituents from the rhizomes of *H. coronarium* for future scientific study of their bioactivities.

Materials and Methods

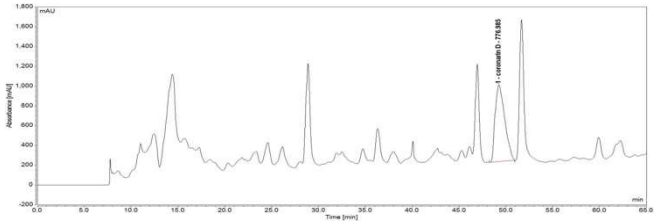
The fresh rhizomes of *H. coronarium* were collected from Ho'omaluhia Botanical Garden in Kaneohe, Oahu and were dried in an oven at 120 degrees for 24 hours. The dried rhizomes were then ground into a fine powder. 158.016 grams of *H. coronarium* powder was mixed with 160 mL of methanol. The mixture was heated under reflux for 24 hours to ensure thorough extraction.

The mixture was filtered and dried using a rotary evaporator. A liquid-liquid extraction using a separatory funnel was performed, using modified Kupchan method, to obtain separate extracts in hexane, chloroform, and ethyl acetate. The three extracts were evaporated under reduced pressure using a rotary evaporator.

The chloroform fraction was subjected to a series of TLC and was separated using reverse phase HPLC with the following time program (Table 1) at 272 nm. The HPLC chromatogram is shown in Figure 1. Four fractions were collected and dried using Savant SpeedVac System. Among the four fractions collected, only fraction #3 was found to be pure.

Table 1. HPLC (Dionex Ultimate 3000 with Phenomenex Luna C18 100A 150 x 4.6 nm column).

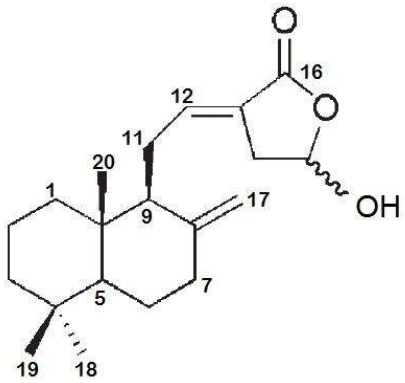
Time (min)	Flow (mL/min)	% water	% acetonitrile
0.00	1.00	50.0	50.0
5.00	1.00	50.0	50.0
55.00	1.00	20.0	80.0
56.00	1.00	50.0	50.0
65.00	1.00	50.0	50.0



Results

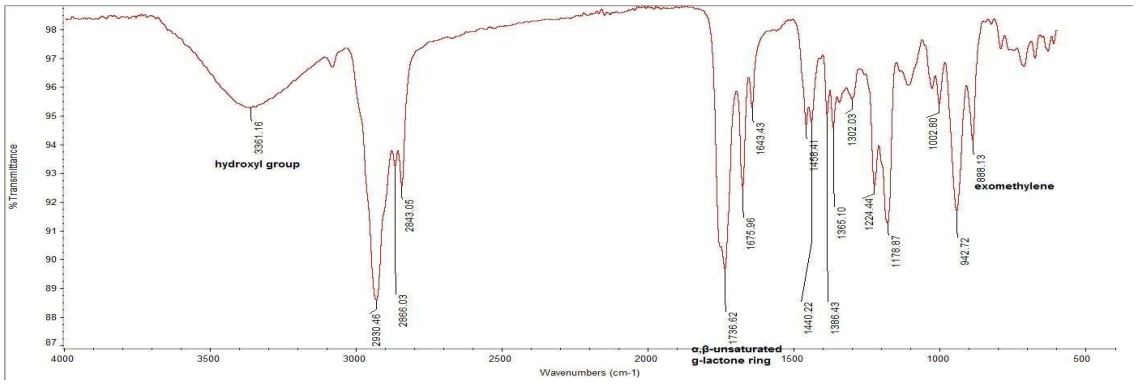
The HPLC fraction #3 was a fragrant, amber colored oil. Its chemical structure was identified as Coronarin D (See Figure 2). This was characterized using UV, FTIR, H-NMR, C-NMR, and mass spectrometry as discussed below.

Figure 2. Structure of compound



The UV-VIS (UV-1800 Shimadzu Spectrometer) spectrum showed a λ max of 223 nm in methanol. The FTIR (Nicolet iS5 FT-IR Spectrometer) spectrum showed absorption bands ascribable to an OH^- group (3361.16 cm^{-1}), an α,β-unsaturated g-lactone C=O group (1736.62 cm^{-1}), a C=C stretch (1643.43 cm^{-1}), and an exomethylene group (888.13 cm^{-1}). See Figure 3.

Figure 3. IR Spectra of compound

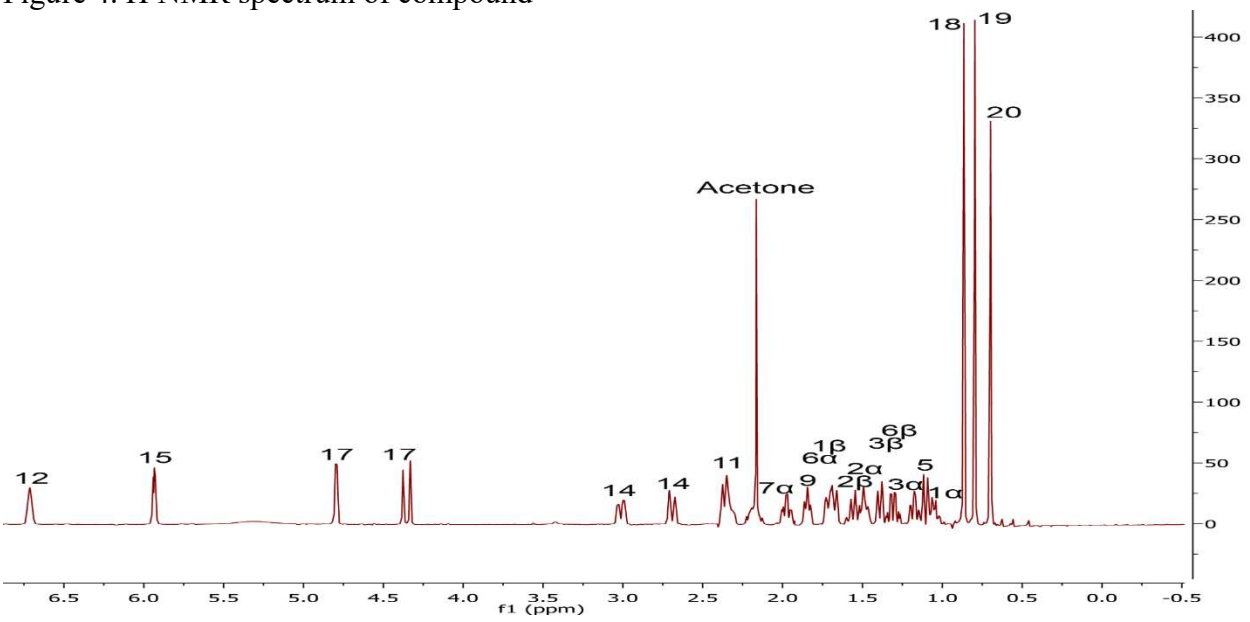


The H NMR spectrum displayed three singlet proton signals at 0.72 ppm, 0.82 ppm, and 0.88 ppm, which were attributed to methyl groups at positions 20, 19, and 18, respectively. The two exomethylene H-17 protons appear as two singlet proton signals, with one set at 4.35 ppm and 4.81 ppm, and another set at 4.40 ppm and 4.83 ppm. The duplicated resonances were attributed to the epimerization located on C-15, as reported in the literature [3]. The chemical shifts of H-11 and H-14 were also affected by the epimerization and showed duplicate chemical shifts. Hence, the isolated compound is actually a mixture of epimers. Analysis of the relative intensities of the H-17 signals indicates that the two epimers were in a 50:50 ratio in the mixture.

Table 2. Comparison of Observed H NMR spectroscopic data of Coronarin D with literature data

H-#	Observed ppm	Chimnoi [3]	Difference	H-#	Observed PPM	Chimnoi [3]	Difference
1α	1.05	1.05	0.00	9	1.84	1.87	0.03
1β	1.70	1.79	0.09	11	2.16 / 2.36	2.20 / 2.39	0.04 /0.03
2α	1.49	1.50	0.01	12	6.71 m	6.75 m	0.04
2β	1.56 ddd (J = 13, 3, 3)	1.58 ddd (J = 13, 3, 3)	0.02	14	2.69 br d (J = 16) / 3.01 m	2.71 br d (J = 17) / 3.04 m	0.02 / 0.03
3α	1.17	1.20	0.03	15	5.93 m	5.93 m	0.00
3β	1.39 (J = 16)	1.41 br d (J = 13)	0.02	17	4.33 s and 4.79 s 4.38 and 4.80 s	One set of 4.35 s / 4.81 s and one set of 4.40 s / 4.83 s	0.02 / 0.02 and 0.02 / 0.03
5	1.10 br d (J = 12)	1.12 br d (J = 13)	0.02	18	0.87 s	0.88 s	0.01
6α	1.72	1.74	0.02	19	0.80 s	0.82 s	0.02
6β	1.31 dd (J = 16, 4)	1.33 dd (J = 13, 4)	0.02	20	0.70 s	0.72 s	0.02
7α	1.97	2.00	0.03				

Figure 4. H NMR spectrum of compound

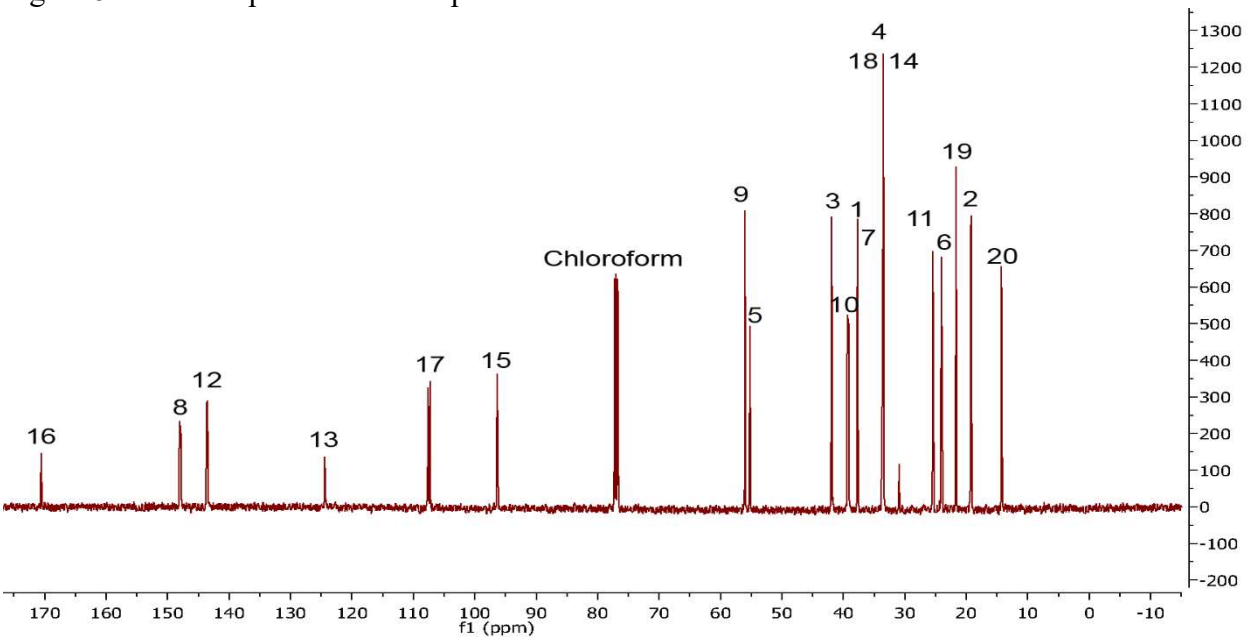


The C NMR spectrum revealed 20 peaks, with duplicated resonances of C NMR at C-8, C-12, and C-17 as shown in Table 3. The duplicated signals showed that the compound was isolated as coronarin D C-15 epimeric mixture. Table 3 compares the C NMR spectroscopic data of the compound with literature value [3].

Table 3. Comparison of Observed C NMR spectroscopic data of Coronarin D with literature data

C-#	Observed ppm	Chimnoi [3]	Difference	C-#	Observed ppm	Chimnoi [3]	Difference
1	39.17	39.23	0.06	11	25.44	25.48	0.04
2	19.24	19.27	0.03	12	143.50 /143.59	143.50 /143.58	0.00 /-0.01
3	41.90	41.96	0.06	13	124.42	124.13	-0.29
4	33.57	33.58	0.01	14	33.50	33.51	0.01
5	55.24	55.33	0.09	15	96.34	95.94	-0.40
6	24.01	24.05	0.04	16	170.57	169.97	-0.60
7	37.70	37.75	0.05	17	107.28/107.56	107.28 / 107.56	0.00 / 0.00
8	147.82/148.03	147.88/148.08	0.06 / 0.05	18	33.63	33.58	-0.05
9	56.03	56.12	0.09	19	21.66	21.67	0.01
10	39.36	39.42	0.06	20	14.28	14.30	0.02

Figure 5. C NMR spectrum of compound



The complete assignments of the protons and carbons were confirmed by the 3-D heteronuclear multiple quantum coherence (HMQC) spectrum, H-H correlation spectroscopy (H-H COSY), and heteronuclear multiple bond correlation (HMBC) experiments. The molecular formula of $C_{20}H_{30}O_3$ was inferred from mass spectrometry ($[M + H]^+$ at m/z 319.2268).

Conclusion

The goal to isolate and characterize a pure compound from *H. coronarium* was accomplished through the isolation of coronarin D. Coronarin D has already been reported to possess cytotoxic activity against cancer cells, antifungal activity against *Candida albicans* and inhibition of both constitutive and inducible nuclear factor-kappa B pathway activation, a mediator of inflammation, apoptosis, invasion, and osteoclastogenesis [2].

Other fractions from the *H. coronarium* will be isolated and purified using different HPLC columns and conditions. It will be very interesting to discover new bioactivities of coronarin D and of the other chemical constituents found in *H. coronarium* in the future.

References

[1] Ajaikumar B. Kunnumakkara, Haruyo Ichikawa, Preetha Anand, Chiramel J. Mohankumar, Padmanabhan S. Hema, Mangalam S. Nair, and Bharat B. Aggarwal. "Coronarin D, a labdane diterpene, inhibits both constitutive and inducible nuclear factor-kappa B pathway activation, leading to the potentiation of apoptosis, inhibition of invasion, suppression of osteoclastogenesis." Mol Cancer Ther 2008; 7(10): 3306-3317.

[2] Nanthawan Reuk-ngam, Nitirat Chimnoi, Nisachon Khunnawutmanotham, and Supanna Techasakul. "Antimicrobial Activity of Coronarin D and Its Synergistic Potential with Antibiotics." BioMed Research International 2014; 1-8.

[3] Nitirat Chimnoi, Somchai Pisutjaroenpong, Lukana Ngiwsara, Decha Dechtrirut, Daranee Chokchaichamnankit, Nisachon Khunnawutmanotham, Chulabhorn Mahidol, and Supanna Techasakul. "Labdane diterpenes from the rhizomes of *Hedychium coronarium*." Natural Product Research 2008; 22(14): 1249-1256.