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ISOLATION OF NEW ROTUNDONE FROM THE ROOTS OF *Croton hirtus* (EUPHORBIACEAE)

(Pemencilan Komponen Rotundona Terbaharu daripada Akar *Croton hirtus* (Euphorbiaceae))

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Abstract

Croton is a genus of Euphorbiaceae which consists of approximately 1,300 species and are widely distributed in Asia, Africa and South America. Phytochemical study on the root of *Croton hirtus* has been conducted. The powder of the root of *C. hirtus* was extracted using the methanol solvent at room temperature for 24 hours and repeated three times. The crude extracts obtained were analysed using thin layer chromatography (TLC) then fractionated *via* vacuum column chromatography (VLC) and proceed the isolation using radial chromatography (RC) to get the pure compounds. The pure compounds obtained were elucidated by nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR) and mass spectroscopy to confirm their structures and from that analysis, the compounds were identified as two new compounds naming (+)5,14-dihydroxyrotundone-9-(2'-methybut-2'-enoate) (**1**) and (-)5,14-dihydroxyrotundane-9-benzoate (**2**) with total weight 135 mg and 145 mg respectively. This two compound were reported for this time on this genus.

Keywords: rotundone, euphorbiaceae, *Croton hirtus*

Abstrak

Croton merupakan genus dalam famili Euphorbiaceae yang mengandungi kira-kira 1,300 spesies dan taburannya meliputi Asia, Afrika dan Amerika Selatan. Kajian fitokimia ke atas akar *Croton hirtus* telah dijalankan. Serbuk akar *C. hirtus* diekstrak dengan menggunakan pelarut metanol pada suhu bilik selama 24 jam dan pengesktrakan diulang sebanyak tiga kali. Ekstrak mentah yang diperoleh dianalisis dengan menggunakan kromatografi lapisan nipis (KLN) kemudian difraksikan melalui kromatografi cecair vakum (KCV) dan pengasingan diteruskan dengan menggunakan kromatografi radial (KR) untuk mendapatkan sebatian tulen. Sebatian tulen yang diperoleh ditentukan oleh resonan magnetik nuklear (RMN), Inframerah transformasi Fourier (TFI) dan spektroskopi jisim untuk mengesahkan struktur mereka dan dari analisis itu, sebatian telah dikenal pasti sebagai dua sebatian baru yang diberi nama (+)5,14-dihidroksirotundona-9-(2'-metibut-2'-enoat) (**1**) and (-)5,14-dihidroksirotundana-9-benzoat (**2**) dengan jumlah berat masing-masing sebanyak 135 mg dan 145 mg. Kedua-dua sebatian ini dilaporkan buat kali pertama bagi genus ini.

Kata kunci: rotundona, euphorbiaceae, *Croton hirtus*

Introduction

Hairy croton or its scientific name *Croton hirtus* are a diverse and largest genus of ornamental plants with about 1,300 species that come from a family of Euphorbiaceae [1, 2]. Mostly, they can be found at Asia, Africa and some of it in North America [3]. Their species include shrubs, trees and herbs that distributed in tropical and subtropical region's ecosystems [4]. Saponins, terpenoids, steroids, phenols, tannins, alkaloids, flavonoids and anthroquinone are the bioactive secondary metabolites that rich in reddish or yellowish latex produced by croton species [5, 6]. Several species also contain aromatic compound, monoterpenoid, diterpenoid, sesquiterpenoids and shikimate – derived compounds due to its volatile oil constituents [7].

Previously, two new compounds from diterpenoid class, (-)5,8-dihydroxyjatrophan-3-one and (+)14,16,17-trihydroxykauran-1-on has been isolated from roots of same species, *Croton Hirtus* [8]. Asia, Africa and South America are among the countries that practiced used this species as their traditional medicine for many application [9, 10]. Biological and pharmacological activities of this genus species have been reported in the past study have played an important role to treat various diseases such as weight loss, anti-inflammatory, diabetes, hepatic disturbances, analgesic, gastrointestinal disturbances, cholesterol level, myorelaxant and cytotoxic [11, 12]. This paper describes NMR study of new rotundone from the roots of *Croton hirtus* species representative from Selangor, Malaysia.

Materials and Methods

The sample used in this research was the stem roots of *C. hirtus* which was collected from Tanjung Sepat, Banting, Selangor, Malaysia. Herbarium specimen of SM 461 was deposited at the Universiti Kebangsaan Malaysia Herbarium.

Extraction and isolation

Dried and ground roots of *C. hirtus* (415 g) were extracted with MeOH to give a dark green extract (18.25 g). The crude extract (18.25 g) was partitioned between $CHCl_3$ and water (1:1) after which the chloroform extract was further extracted with 10% *n*-hexane and 90% MeOH to give MeOH sub-extract. The sub-extract (3.52 g) was fractionated by using silica gel vacuum liquid chromatography eluted with increasing polarity of *n*-hexane-EtOAc to give ten fractions. The eluents that showed the same profile on thin layer chromatography (TLC) were combined to give four fractions (I-IV). Purification of fraction II (0.2027 g) was carried out using radial chromatography (RC) with silica gel plate of 1 mm thickness eluted with 60:40 *n*-hexane-EtOAc in 5% polarity increment to yield compound 1 (135 mg). Purification of fraction III (0.3163 g) was also carried out in a similar manner eluting with 1:1 *n*-hexane-EtOAc producing sub-fractions III^a-III^f. Sub-fraction (III^c= 0.0221 g) was further purified using RC with silica gel plate of **1** mm eluting with 30:70 *n*-hexane-EtOAc which resulted in the isolation of compound **2** (145 mg).

Analysis

The isolated compounds (**1** and **2**) were elucidated by means of Perkin-Elmer Lambda 35 UV-Vis (Waltham, MA, USA), Perkin-Elmer FT-IR (Waltham, MA, USA), Agilent Technologies GC-MS (Santa Clara, CA, USA) and one and two dimensioned nuclear NMR (Bruker, Billerica, MA, USA) spectroscopy. The proton NMR was recorded in 400 MHz while the carbon in 100 MHz. Chemical shift, δ, ppm recorded in $CDCl_3$.

Results and Discussion

(+)5,14-dihydroxyrotundone-9-(2'-methybut-2'-enoate**)** (**1**) was isolated as a clear needle crystal with molecular formula $C_{20}H_{32}O_4$, obtained by HR-CI–MS $[M+1]^+$ 337.2276 m/z, m.p. 123-125 $^{\circ}$C, and $[\alpha]_D^{25}$ +16.15 (*c* 1.30, $CHCl_3$). The IR spectra exhibited absorptions for hydroxyl groups (3347 cm^{-1}), cyclic aliphatic group (2958 and 2875 cm^{-1}), carbonyl group (1697 cm^{-1}) and double bond (1499 cm^{-1}).

The ^{13}C spectrum indicated the presence of 20 carbons consisting of four methyls, six methylenes, six methines and four quaternary carbons. The most highly deshielded signal at δ_C 168.1 (C-1`) integrated for carbon attributed to a carbonyl group, two signals for olefinic carbons at $\delta_C$ 128.8 (C-2`) and 137.3 (C-3`) are linked with two methyl carbons at $\delta_c$ 14.4 (C-4`) and 12.1 (C-5`), while the other five methine carbons at δ_c 59.8 (C-1), 49.1 (C-4), 44.4 (C-7), 76.5 (C-9) and 37.1 (C-11) belongs to five methylene carbons at δ_c 32.0 (C-2), 28.4 (C-3), 49.7 (C-6), 25.4 (C-8)

and 32.8 (C-2) are indicative of the aliphatic component of the cyclic system. The DBE value of $C_{20}H_{32}O_4$ is 5, indicating that the compound consists of two double bonds (one olefinics and one carbonyl) and three rings of aliphatic group. The ^{1}H-NMR spectrum showed 22 signals representing 32 protons. Twenty-two of the signals came from the three signals, four each from methyl group at H-4` (1.80, *d*, 7.0, 3H) and H-5` (1.84, *s*, 3H) bonded to an olefinic carbon at C-2` and C-3`, while the other two protons at H-13 (0.91, *d*, 6.6, 3H) and H-15 (0.89, *d*, 6.6, 3H) represent the two non- equivalent methyl protons in the cyclic aliphatic.

Furthermore, twelve signals at H-2 (2.03, *dd*, 14.0, 4.0, 1H & 1.42, *m*, 1H), H-3 (1.13, *m*, 1H & 1.89, *m*, 1H), H-6 (1.74, *d*, 7.0, 1H & 1.31, *m*, 1H), H-8 (2.20, *ddd*, 12.8, 12.4, 4.9, 1H & 1.75, *dd*, 12.8, 4.9, 1H), H-12 (1.50, *d*, 7.0, 1H & 1.22, *d*, 7.0, 1H) and H-14 (4.04, *d*, 11.0, 1H & 3.43, *d*, 11.0, 1H) represent the six non-equivalent methylene protons in the cyclic aliphatic while the other five signals at H-1 (1.46, *m*, 1H), 1.60, *m*, 1H), H-7 (0.79, *m*, 1H), H-9 (4.92, *dd*, 12.4, 4.9, 1H) and 1.15, *m*, 1H) represent the five methine protons (Figure 1).

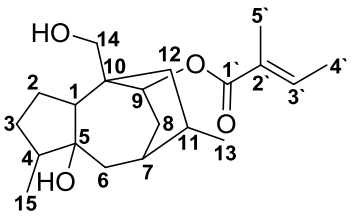

Figure 1. Chemical structure of **1**

The structure of the compound **1** was further confirmed using 2D NMR experiments including HMBC and COSY. From the HMBC experiments, the position of carbonyl carbon (C-1`) was proven by correlations of C-3` and C-9 toward C-1`. The proton H-15 in correlation with C-5 confirmed the position of this carbinol group bonded to C-5 and C-14 as branches to another one carbinol group. The H-H COSY experiment indicated that protons at δ_H 6.48 (1H, *dq*, *J*= 1.4, 7.0) and 1.80 (3H, *d*, *J*= 7.0) were related to each other and marked as olefinic (C-3`) and methyl protons at C-4`. Furthermore, correlation between saturated methines and methylenes protons was forming rotundone skeleton as main structure in this molecule (Figure 2).

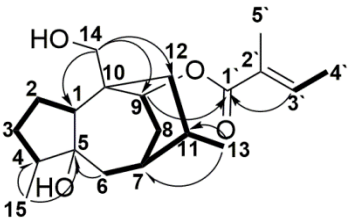

Figure 2. HMBC (→) and H-H Cosy (—) Correlation
Compound **1**

(-)5,14-dihidroxyrotundone-9-(2'-methylene-3'- hidroxybutanoate) (**2**) was isolated as pink olily and o.r. $[\alpha]_D^{25}$ - 27.14 (*c* 1.40, $CHCl_3$). The molecular formula is $C_{20}H_{32}O_5$, generated using HR-ESI-MS $[M + H]^+$ ion at m/z: 353.2217 (calc. for $C_{20}H_{32}O_5$ 353.2217). The FTIR spectral data displays broad absorbance peak at 3326 representing hydroxyl, 2985 and 2881 cm-1 representing the C-H (aliphatic cyclic). The presence of carbonyl group is characterized by absorbance bands, i.e. C=O stretch at 1716 cm^{-1} indicated that the carbonyl group linked with oxygen forming ester. Furthermore, Csp^2-H is characterized by absorbance at 1454-1401 cm^{-1}.

The ^{13}C spectrum contains 20 carbons consisting of three methyl, seven methylenes, six methines and four quaternary carbons. The most highly deshielded signals at δ_c 166.6 (C-1`) is attributed to carbonyl group linked with

another oxygen was formed ester group. Two signals of methine at δ_c 77.4 (C-9) provide information of the presence of a 2'-methylene-3'-hidroxybutanoate, while the other one (C-4) bind with an equivalent proton methyl (C-15). Carbons C-5, C-3` and C-14 represent the existence of carbinol groups in the molecule. The DBE value of $C_{20}H_{32}O_5$ is 5, indicating that the compound consists of two double bonds as carbonyl group and an olefinic group branch. Meanwhile, 23 proton signals representing 32 protons appeared in the ^{1}H-NMR spectrum. Twenty three of the signals came from the three signals, one each from a methyl group at H-4` (3H, 1.37, *d, J*= 6.3) bind with an oxymethine proton at H-3` (δ_H 4.96) are quartet, while an olefinic non-equivalent methylene protons at C-14. Furthermore, the other methine at δ_H 1.53 and 1.30 are multiplet, indicating it is adjacent to any carbons contain more than 4 total protons. The HMBC and H-H Cosy correlation of compound **2** show in Figure 3.

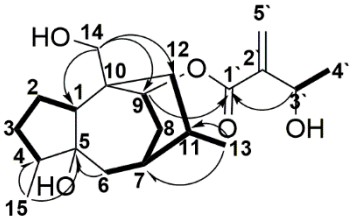

Figure 3. HMBC (→) and H-H Cosy (—) Correlation
Compound **2**

Conclusion

Two sesquiterpenoids; (+)5,14-dihydroxyrotundone-9-(2'-methybut-2'-enoate**)** (**1**) and (-)5,14-dihidroxyrotundone-9-(2'-methylene-3'- hidroxybutanoate) (**2**) were successfully isolated from the methanolic extract of *Croton hirtus*. To the best of our knowledge, this is the first report for both compounds from this species. Both of compounds can be used to carry out biological activities because this species was known as many useful in traditional medicine.

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