บทความวิจัย

แซนโทนที่พบในยางผลมังคุด

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บทคัดย่อ

งานวิจัยนี้ศึกษาองค์ประกอบทางเคมีของยางผลมังคุด ผลจากการศึกษาพบว่ายางผลมังคุดมีแซนโทน 13 สาร ในขณะที่เคยมีผู้รายงานไว้แล้วเพียง 5 ชนิด และเป็นครั้งแรกที่รายงานการแยก cowaxanthone D และ cratoxylone ในพืชสกุลนี้ การพิสูจน์โครงสร้างของแซนโทนทั้งหมดใช้ 1D และ 2D นิวเคลียร์แมกเนติกเรโซแนนซ์เทคนิก และเปรียบเทียบข้อมูลกับสารที่มีผู้รายงานไว้แล้ว

คำสำคัญ: แซนโทน ยางผลมังคุด คราทอกไซโลน ไอโซเมอร์โครงสร้าง มังคุด

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Xanthones of the *Garcinia mangostana* Fruit Latex

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ABSTRACT

This research studied on the chemical constituents of fruit latex from *Garcinia mangostana* (L). The result showed that the fruit latex contained 13 prenylated xanthones, whereas only five xanthones have been reported from previous work. In addition, this is the first report on the isolation of cowaxanthone D and cratoxylone from this plant species. The structures of all isolated compounds were elucidated mainly by 1D and 2D NMR spectroscopic techniques and comparisons of their spectroscopic data with the literature values.

Keywords: xanthone, latex of mangosteen, cratoxylone, structural isomer, mangosteen

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Introduction

The mangosteen fruit is originated from Southeast Asia, including Thailand [1], and is well known as Queen of fruit [2]. Mangosteen tree requires warm and very humid, climate year round. Mangosteen or *Garcinia mangostana* L. (family Clusiaceae) has been used as a traditional medicine for treatment of skin infection and wounds in Southeast Asia for many years and is a major economic fruit of Thailand. The fruit pericarp contains high amount of prenylated xanthones with a wide range of biological activities, such as anticancer [3], antibacterial [4], anti-inflammatory [5], antioxidant [6] and cardiovascular activities [7]. Phytochemicals studies on *G. mangostana* fruit, stem, leaf and root have been published extensively, however, only two groups worked on the fruit latex and only five xanthone compounds were reported from this plant part [8, 9]. Dharmaratne et al. revealed that the green fruit latex consists of more than 75% xanthones and hence was another rich source of xanthones [9]. Mangosteen is becoming a major botanical dietary supplement and its sales have been greatly increased in the U.S. and around the world because of the rather high levels of xanthones in mangosteen juice and their purported health benefits [10].

Based on its various health benefits and only a limited number of reports on the fruit latex, in this study the EtOAc-soluble extract obtained from the fruit latex of *G. mangostana* was subjected to chemical investigation leading to the isolation and identification of thirteen xanthones (**1-13**, Figure 1), of which cowaxanthone D (**9**) and cratoxylone (**13**) are reported from this plant species for the first time. Isolation of two structural isomers, **12** and **13**, was also described.

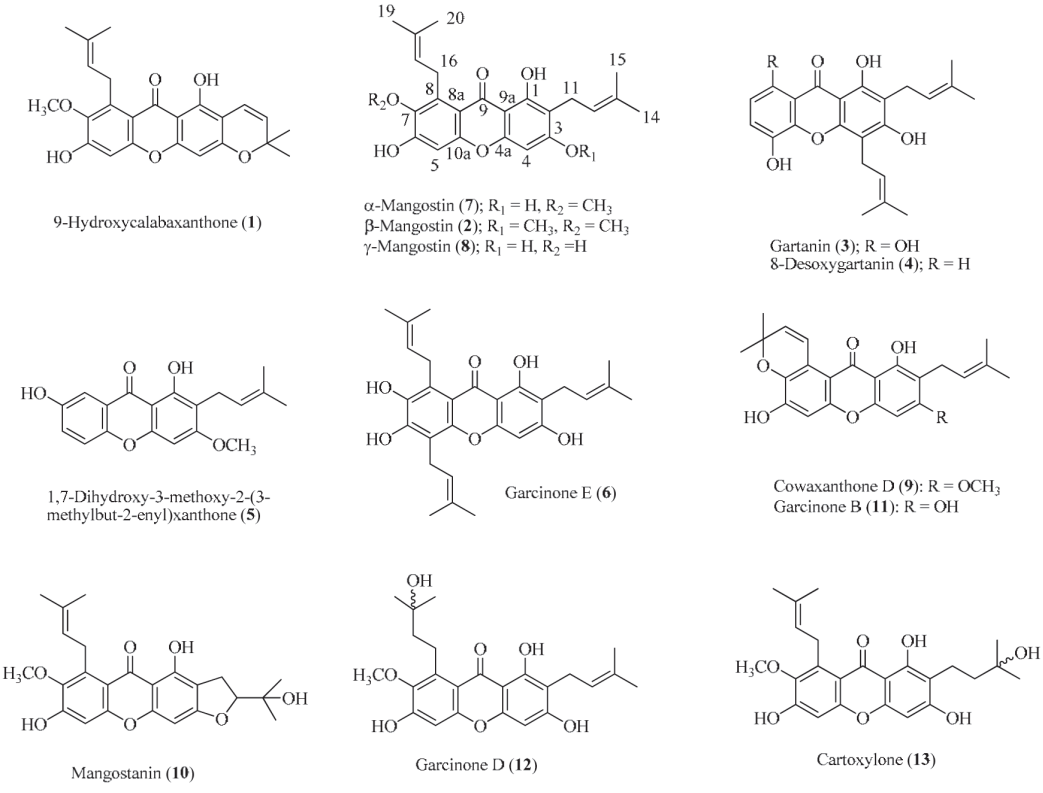


Figure 1 Structures of 13 xanthones isolated from the fruit latex of *G. mangostana*

Materials and Method

Source of Plant Material

The dried yellow fruit latex of *G. mangostana* was collected from the fruits purchased from local market in Bangkok in July 2008.

General Procedures

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 FT-NMR spectrometer, operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). The chemical shifts were referenced to the residual solvent peaks (δ_H 7.24 and δ_C 77.00 for $CDCl_3$, δ_H 2.04 and δ_C 29.8 for acetone-d_6). Column chromatography and thin layer chromatography (TLC) were carried out using Merck silica gel 60 (> 230 mesh) and precoated silica gel 60 F254 plates, respectively. Plates of silica gel PF254, 2.00 mm thickness, were utilized for centrifugal TLC. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating. Melting points, in degree Celsius, were measured on a Griffin melting point apparatus. Electrospray ionization (ESI) mass spectra were obtained on a Finnigan LC-Q mass spectrometer.

Extraction and isolation

The dried fruit latex (17 g) including a small amount of pericarp material was dissolved with ethyl acetate (3 × 300 ml) and filtered. Evaporation of the filtrate under reduced pressure gave a brown paste (15.3 g) which was separated into 6 major fractions by chromatography on a silica gel column, eluting sequentially with *n*-hexane, *n*-hexane-acetone (96:4-50-50) and acetone. Fraction 2 (3.24 g) was subjected to silica gel column chromatography employing a gradient of *n*-hexane-acetone (98:2-90:10) resulting in 10 subtractions. Subfraction 2.5 yielded compound **1** (248.3 mg). Compound **2** (7.1 mg) was isolated from subfraction 2.7 by precipitation in *n*-hexane and the yellow solid was washed with cold *n*-hexane. Subfraction 2.9 (164.7 mg) was further purified by silica gel column chromatography employing a gradient of *n*-hexane-acetone (100:0-97:3) to obtain a mixture of compounds **3** and **4** (7.8 mg) and compound **5** (9.0 mg). Subfraction 2.10 (213.0 mg) was rechromatographed over silica gel with *n*-hexane-acetone (95:5-90:10) as the solvent system to afford compound **6** (17.3 mg). The major compound **7**, α-mangostin, precipitated as a yellow solid (8.7 g) when fraction 3 was left in 8% acetone-*n*-hexane overnight. Compound **8** (1.6 g) similarly precipitated from fraction 4 (6.57 g, 12% acetone-*n*-hexane). Subfraction 4.9 (166.9 mg) was fractionated by centrifugal thin-layer chromatography (Silica gel 60 PF_{254} containing $CaSO_4$, plate of 2.0 mm thickness) and eluted with *n*-hexane-ethyl acetate of increasing polarity (95:5-85:15) to provide 6 subfractions (4.9a-4.9f). Compounds **9** (2.9 mg) and **10** (4.7 mg) were obtained from subfractions 4.9a (2.9 mg) and 4.9e (44 mg), respectively, as pale yellow solids. Subfraction 4.11 (152.1 mg) was fractionated by silica gel column chromatography eluted with dichloromethane-methanol (99:1-85:15) as eluent to provide 15 further subfractions (4.11a-4.11o). Subfraction 4.11b (0.5 mg) gave compound **11** as a pale yellow solid. A mixture of garcinone D and cratoxylone, obtained from fraction 5 (6.33 g), was subjected to silica gel column chromatography employing solvent a gradient *n*-hexane-acetone (90:10-80:20) and 8 subfractions were collected. Subfraction 5.6 (560.3 mg) was further chromatographed over a silica gel column using *n*-hexane-acetone (90:10-80:20) as eluting solvent to provide compound **13** (39.3 mg) as a pale yellow solid. Compound **12** (119.9 mg) was obtained from subfraction 5.7 (543.6 mg) as a pale yellow solid.

Table 1 ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data of compounds **9** and **13**

Position	Compound 9[a]		Compound 13[b]	
	δ_H	δ_C	δ_H	δ_C
1-OH	13.31 (1H, s)	159.4	13.78 (1H, s)	161.6
2		111.2		112.3
3		163.4		163.2
3-OMe	3.84 (3H, s)	55.7		
4	6.29 (1H, s)	88.9	6.36 (1H, s)	93.3
4a		155.4		155.6
5	6.73 (1H, s)	102.3	6.81 (1H, s)	102.6
6		151.2		157.3
7		137.2		144.4
7-OMe			3.78 (3H, s)	61.2
8		119.8		138.0
8a		108.5		111.8
9		ca 182.1		182.8
9a		103.8		103.4
10a		152.9		156.1
11	3.29 (2H, br d, J = 7.2 Hz)	21.2	2.74 (2H, dt, J = 5.3, 7.9 Hz)	18.2
12	5.15 (1H, br t, J = 7.2 Hz)	122.1	1.69 (2H, dt, J = 5.3, 7.9 Hz)	43.1
13		131.6		70.6
14	1.72 (3H, s)	17.6	1.24 (3H, s)	29.0
15	1.61 (3H, s)	25.6	1.24 (3H, s)	29.0
16	7.95 (1H, d, J = 10.2 Hz)	120.9	4.11 (2H, d, J = 6.3 Hz)	26.8
17	5.75 (1H, d, J = 10.2 Hz)	132.1	5.26 (1H, t, J = 6.3 Hz)	124.7
18		76.4		131.3
19	1.43 (3H, s)	27.0	1.81 (3H, s)	25.8
20	1.43 (3H, s)	27.0	1.63 (3H, s)	17.8

Note: [a]in $CDCl_3$

[b]in acetone-d_6

ca approximately

Results

TLC observation of the ethyl acetate extract of the *G. mangostana* fruit latex, showed the green-yellow spots after development with anisaldehyde-H_2SO_4 reagent, indicative of the presence of prenylated xanthones. The ethyl acetate soluble fraction of the fruit latex was further purified to yield thirteen xanthones (**1-13**), whereas only *α*-mangostin, *γ*-mangostin, *β*-mangostin and garcinone E were obtained in previous works on the fruit latex of this plant [8, 9]. In addition, this is the first report on the isolation of cowaxanthone D (**9**) and cratoxylone (**13**) from *G. mangostana.*

Cowaxanthone D (**9**): Pale yellow solid; m.p. 222-224 °C (lit. 210.0-210.7 °C [11]); UV λmax MeOH nm (log ε): 245 (4.44), 265 (4.42), 328 (4.30), 387 (3.81); IR (KBr) cm^{-1}: 3483 (O-H), 1645 (C=O); ^{1}H- and ^{13}C-NMR data: see Table 1; ESIMS (-ve): *m/z* (% relative intensity): 407.3 $[M-H]^-$ (100).

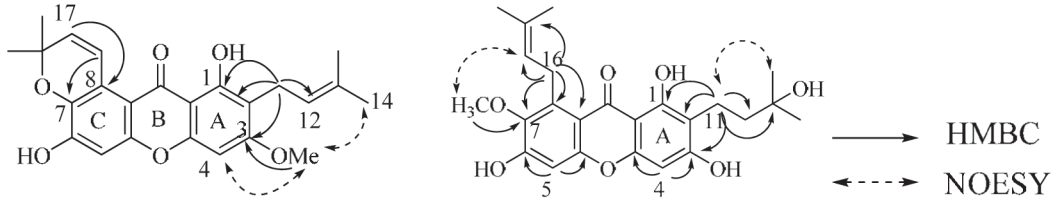
Cratoxylone (**13**): Pale yellow solid; m.p. 229-230 °C; UV λmax MeOH nm (log ε): 242 (4.24), 256 (4.15), 316 (4.06 × 348 (3.72). IR (KBr) cm^{-1}: 3408 (O-H), 1646 (C=O); ^{1}H- and ^{13}C-NMR data: see Table 1; ESIMS (+ve): *m/z* (% relative intensity): 427.4 $[M-H]^-$ (80).

Discussion and conclusion

Phytochemical study of the *G. mangostana* fruit latex led to the first isolation of two xanthones, cowaxanthone D (**9**) and cratoxylone (**13**), from this plant species along with eleven known xanthones, including 9-hydroxycalabaxanthone (**1**) [13], *β*-mangostin (**2**) [15], gartanin (**3**) [14], 8-desoxygartanin (**4**) [14], 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone (**5**) [14], garcinone E (**6**) [16], *α*-mangostin (**7**) [17], *γ*-mangostin (**8**) [17], mangostanin (**10**) [18], garcinone B (**11**) [19] and garcinone D (**12**) [13] (Figure 1). The structures of these compounds were elucidated mainly by NMR spectroscopic techniques and comparisons with the previously reported data.

Compound **9** was obtained as a pale yellow solid and showed a pseudomolecular ion peak at *m/z* 407.3 $[M-H]^-$ in its ESIMS, corresponding to the molecular formula $C_{24}H_{23}O_6$. The IR spectrum showed absorption bands at 3483 cm^{-1} for one or more hydroxyl group and 1645 cm^{-1} for a carbonyl functionality. The ^{1}H NMR spectrum (Table 1) obtained in $CDCl_3$ revealed signals for a 1,3,6,7-tetraoxygenated xanthone, which include one chelated hydroxyl group at δ_H 13.31, two aromatic singlets at δ_H 6.29 (H-4), δ_C 88.9 and δ_H 6.73 (H-5), δ_C 102.3; a 3-methylbut-2-enyl group at δ_H 5.15 (1H, br t, J = 7.2 Hz, H-12), δ_C 122.1 (C-12); δ_H 3.29 (2H, d, J = 7.2 Hz, H-11), δ_C 21.2 (C-11); δ_H 1.72 (s) (3H, s, H-14), δ_C 17.6 (C-14) and δ_H 1.61 (s) (3H, s, H-15), δ_C 25.6 (C-15) and a 2,2-dimethylchromene ring at δ_H 7.95

(1H, d, J = 10.2 Hz, H-16), δ_C 120.9 (C-16); δ_H 5.75 (1H, d, J = 10.2 Hz, H-17), δ_C 132.1 (C-17) and δ_H 1.43 (6H, s, H-19, H-20) , δ_C 27.0 (C-19, C-20). This 2,2-dimethylchroman ring was attached at C-7 (δ_C 137.2) and C-8 (δ_C 119.8) base on an anisotropic effect of the C-9 carbonyl group (δ_C 182.1) which caused a downfield shift of H-16 signal. In addition, the two olefinic protons at δ_H 7.95 and 5.75 of the 2,2-dimethylchroman ring displayed correlations with C-7 (δ_C 137.2) and C-8 (δ_C 119.8), respectively, in its HMBC experiment (Figure 2). Compound **9** was then shown to be a close analogue, but less polar (with R_f value of 0.54; 30% acetone in *n*-hexane) than garcinone B (**11**). The difference could readily be observed in the ^{1}H NMR spectrum (Table 1) in which an additional methoxyl singlet signal at δ_H 3.84, δ_C 55.7 was observed. The location of this methoxyl group at C-3 (δ_C 163.4) was confirmed by its HMBC correlations (Figure 2) with C-3. NOE enhancement (Figure 2) with aromatic proton H-4 (δ_H 6.29) and methyl proton H-14 (δ_H 1.61) in the NOESY spectrum, further confirmed the attachment of this group at C-3. Thus, the structure of **9** was assigned to be 1,6-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano(2′,3′:7,8)xanthone or cowaxanthone D, a xanthone previously isolated from the fruits of *G. cowa* [11].



9 **13**

Figure 2 Selected HMBC and NOESY correlations of cowaxanthone D (**9**) and cratoxylone (**13**)

^{1}H NMR spectrum of fraction 5 showed a mixture of two compounds (**12** and **13**) although it gave only one green spot on TLC in various eluting solvent system. Nevertheless, compound **13** was successfully isolated by silica gel column chromatography technique as a pale yellow solid that showed a pseudomolecular ion peak at *m/z* 427.4 $[M-H]^-$ in the ESIMS which corresponds to a molecular formula of $C_{24}H_{28}O_7$. IR spectrum of **13** exhibited the same pattern as that of garcinone D (**12**). 1D and 2D NMR data analysis of compound **13** showed that this xanthone has the same two aromatic protons and substituents as those of garcinone D (**12**), including a 3-hydroxy-3-methylbutyl, a prenyl, one chelated hydroxyl and one methoxyl groups (Table 1). This suggested that compound **13** was an isomer of **12**. Comparison of their ^{1}H NMR spectra (Figure 3) suggested their differences were in the placement for the 3-hydroxy-3-methylbutyl and prenyl moieties. From HMBC spectral data

of **13** (Figure 2), the methylene proton signal at δ_H 2.74 (2H, dt, J = 5.3, 7.9 Hz, H-11) of the 3-hydroxy-3-methylbutyl group showed correlations with C-1 (δ_C 161.6), C-2 (δ_C 112.3) and C-3 (δ_C 163.2). It was therefore apparent that this side-chain was located at C-2 (δ_C 112.3) in ring A. The downfield methylene proton signal of the prenyl unit at δ_H 4.11 (H-16) showed correlations with C-7 (δ_C 144.4), C-8 (δ_C 138.0) and C-8a (δ_C 111.8) and the methoxyl group at δ_H 3.78 (7-OMe) showed correlations with C-7 (δ_C 144.4). In addition, the methoxyl proton at δ_H 3.78 also exhibited a NOESY correlation (Figure 2) with the olefinic proton H-17 (δ_H 5.26). This confirmed the positions of a prenyl and a methoxyl moiety in **13** at C-8 and C-7, respectively. From these evidences, the structure of compound **13** was concluded to be 1,3,6-trihydroxy-2-(3-hydroxy-3-methylbutyl)-7-methoxy-8-(3-methylbut-2-enyl)xanthone or cratoxylone. Cratoxylone has been isolated from the bark of *Cratoxylum cochinchinense* [12].

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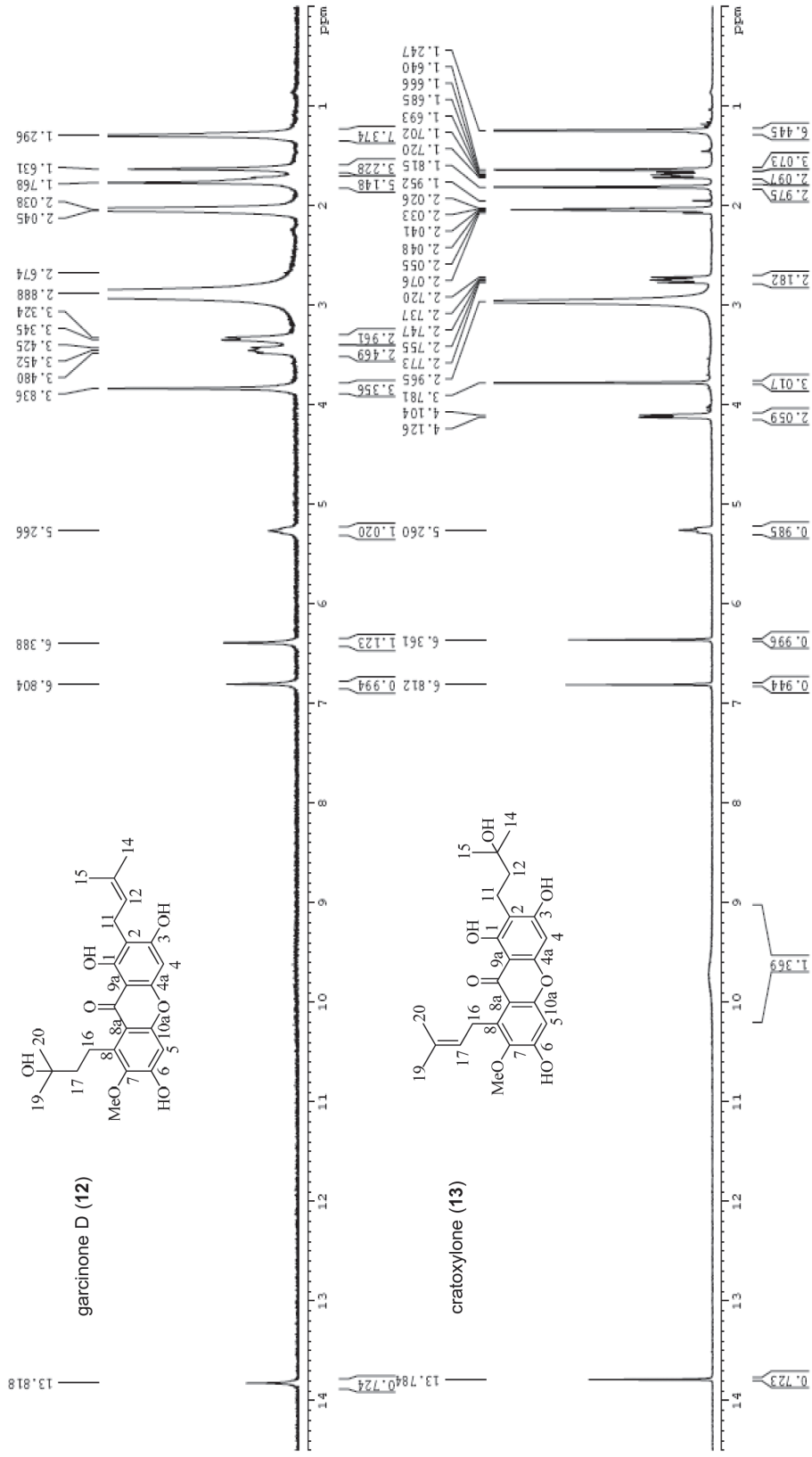

Figure 3 ^{1}H NMR (300 MHz) spectra of garcinone D (**12**) and cratoxylone (**13**)

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