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XANTHONES FROM THE TWIGS OF THE VIETNAMESE *CALOPHYLLUM CERIFERUM* GAGNEP. EX STEVENS

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ABSTRACT

Three xanthones have been isolated from the dichloromethane extract of the twigs of the Vietnamese *Calophyllum ceriferum* Gagnep. Ex Stevens collected at Ninh Thuan province. Their structures were elucidated as 1,7-dihydroxy-2,3,8-trimethoxyxanthone, 1,7-dihydroxyxanthone and 1,6-dihydroxy-5,7-dimethoxyxanthone by spectroscopic analyses.

Keywords: Calophyllum ceriferum, 1,7-dihydroxy-2,3,8-trimethoxyxanthone, 1,7-dihydroxyxanthone, 1,6-dihydroxy-5,7-dimethoxyxanthone.

1. INTRODUCTION

Calophyllum genus belongs to Clusiaceae family. According to the literature [1], in Vietnam the *Calophyllum* genus is reported to consist of 15 different species, most of which are wild growing. Our previous phytochemical investigations of the Vietnamese *Calophyllum* species reported the chemical constituents of two species *C. inophyllum* (cây Mù u) and *C. balansae* (cây Rù ri) including coumarins, triterpenes, flavones and xanthones [2, 3, 4]. *C. ceriferum* is wild growing at Ninh Thuan province, which was collected by the botanists of Institute of Ecology and Biological Resources. In this context, we report on the isolation and characterization of three xanthones 1,5-dihydroxy-2,3,8-trimethoxyxanthone (**1**), 1,7-dihydroxyxanthone (**2**) and 1,6-dihydroxy-5,7-dimethoxyxanthone (**3**) from the dichloromethane extract of the twigs of *Calophyllum ceriferum.*

2. EXPERIMENTAL SECTION

General Experimental Procedures: Melting points were measured with a Büchi B-545 apparatus. EIMS were measured with a HP 5989B mass spectrometer. NMR spectra were recorded by a Bruker Avance 500 MHz instrument using TMS as internal standard.

Plant material: The twigs of *Calophyllum ceriferum* were collected at Ninh Thuan province and identified by Dr. Nguyen Tien Hiep, Institute of Ecology and Biological Resources, where a

voucher specimen is deposited.

Extraction and Isolation: The dried and powdered material (210 g) was extracted with MeOH at room temperature (3 times, 2 days/time). After a part of MeOH was evaporated *in vacuo*, the MeOH-solution was extracted successively by n-hexane and dichloromethane to obtain n-hexane extract (**F1**, 4 g) and dichloromethane extract (**F2**, 2 g). **F2** was subjected to column chromatography on silica gel, eluted with gradient n-hexane-dichloromethane and dichloromethane-methanol to obtain 4 fractions **F3-F6**. Then **F4** continue to be seperated by column chromatography on silica gel, eluted with n-hexane-dichloromethane 50 % to obtain 5 fractions **F7-F11**. Further separation of **F8** by gel filtration on Sephadex LH-20 with MeOH-CH_2Cl_2 8:2 yielded a main fraction, which was crystallized in acetone to obtain compound **3** (2mg). **F9** and **F10** were filtrated on Sephadex LH-20 with MeOH-CH_2Cl_2 8 - 2 to yield 2 fractions **F12-F13**. Crystallization of F**12** in acetone yielded xanthone **1** (2 mg). **F13** was crystallized in acetone to obtain xanthone **2** (5 mg).

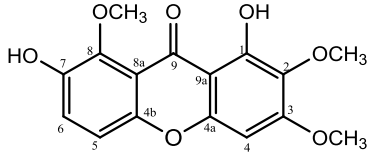
1,7-dihydroxy-2,3,8-trimethoxyxanthone (**1**): yellow needles (n-hexane-acetone), mp. 204 ^{0}C. TLC: R_f 0.3 (n-hexane/acetone 7/3). EIMS *m/z* (%): 318 ($[M]^+$, 100), 303 ($[M\text{-}CH_3]^+$, 58), 275 (59), 260 (75), 245 (45), 153 (24), 93 (26), 69 (19). ^{1}H and ^{13}C NMR see table 1.

1,7-dihydroxyxanthone (**2**): yellow crystal (n-hexane-acetone), mp. 235-236 ^{0}C. TLC: R_f 0.46 (n-hexane/acetone 7/3). EIMS *m/z* (%): 228 ($[M]^+$, 100), 200 (15), 171 (5), 144(8), 115 (16), 57 (21). ^{1}H and ^{13}C NMR see table 1.

1,6-dihydroxy-5,7-dimethoxyxanthone (**3**): yellow crystal (n-hexane-acetone), mp. 215-216. TLC: R_f 0.4 (n-hexane/acetone 7/3). ^{1}H and ^{13}C NMR see table 1.

3. RESULTS AND DISCUSSION

From the dichloromethane extract of the twigs of *C. ceriferum*, 3 xanthones have been isolated on the basis of chromatographic methods.



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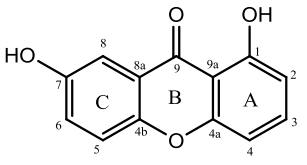

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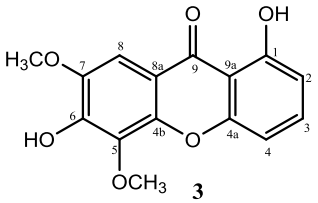

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Xanthone **1** was obtained as yellow needles, mp 204 ^{0}C, which gave a HREIMS (High Resolution Electron Impact Mass Spectroscopy) parent ion at *m/z* 318.0789 Da indicating a molecular formula of $C_{16}H_{14}O_7$. ^{13}C NMN spectrum showed the resonance of 16 carbons corresponding to 3 methyl, 3 methine groups and 10 aromatic quaternary carbons including a conjugated carbonyl (δ_C 180.90). The typical signals at δ_C 180.90 (C-9), 152.96 (C-4a), 150.76 (C-4b), 114.51 (C-8a) and 104.45 (C-9a) revealed a xanthone skeleton for this compound. The ^{1}H NMR spectrum showed the presence of a chelated hydroxyl group (δ_H 13.02), a phenolic hydroxyl group (δ_H 6.00) and three aromatic protons appearing as two doublets at δ_H 7.38 and 7.16 (2H, d, *J*=9.0 Hz, H-8, H-7) and a singlet at δ_H 6.42, which belonged to two distinct benzene rings. These data indicated a pentasubstituted xanthone skeleton for this compound. The long- range couplings in the HMBC spectrum between chelated hydroxyl proton with C-2, C-9a and C-1 allowed to prove that this hydroxyl group was located at C-1. Correlations between the protons of 3 methoxyl groups with C-2, C-3 and C-8 indicated the position C-2, C-3 and C-8 of these groups, respectively. Correlations between the remaining phenolic hydroxyl proton with

C-8, C-7 and C-6 clearly proved the position C-7 of this group. Full analyses of 1D and 2D NMR spectra allowed to establish the structure of this compound as 1,7-dihydroxy-2,3,8-trimethoxyxanthone.

The ^{13}C NMR of **2** exhibited 13 carbon signals assigned by DEPT experiment to 6 methine groups, 4 oxygenated quaternary carbons, one carbonyl group and 2 other quaternary carbons. This together with the molecular peak at *m/z* 228 in the EIMS spectrum corresponding to a molecular formula of $C_{13}H_8O_4$. The ^{13}C NMR data also suggested a xanthone skeleton for this compound. The ^1H NMR spectrum showed resonance signals of a chelated hydroxyl proton (δ_H 13.02), a phenolic hydroxyl proton (δ_H 6.00) and 6 aromatic protons appearing as one ABX and one AB_2 spin coupling systems. The first system AB_2 composed of three protons appearing at δ_H 6.71, 6.87 (2H, dd, *J* = 8.5, 0.5 Hz, H-2, H-4) and 7.52 (t, *J*= 8.5, H-3), which indicated the presence of a 1,2,3-trisubstitued benzene ring (ring A). The presence of a 1,2,4-trisubstituted benzene ring (ring C) was deduced from the second coupling system ABX of three protons at δ_H 7.33 (d, *J* = 9.0 Hz, H-5), 7.25 (dd, *J* = 9.0, 3.0, H-6) and 7.47 (d, *J* = 2.5 Hz, H-8). These spectra evidences revealed a disubstituted xanthone skeleton for this compound. The position of two hydroxyl groups were established by the correlations in the HMBC spectrum at C-1 and C-7. By combined analysis of the spectra data, compound **2** was identified as 1,7-dihydroxyxanthone. This compound have been first isolated from *Hypericum mysorense* [5] and *Mesua thwaitesii* [6].

Xanthone **3** was isolated as yellow crytals, mp 215 - 216 ^0C. Molecular formula of **3** was deduced to be $C_{15}H_{12}O_6$ by the ^{13}C NMR spectra data. The ^{13}C NMR had resonance signals of 15 carbons corresponding to 2 methyl, 4 methine and one carbonyl groups, 6 oxygenated quaternary carbons and 2 other quaternary carbons. The ^1H NMR spectrum showed the presence of 4 aromatic protons as a singlet at δ_H 7.44 and a AB_2 coupling system of three protons at δ_H 6.80, 7.00 (2 dd, *J* = 8.5, 0.5 Hz, H-2, H-4) and 7.00 (t, *J* = 8.5 Hz, H-3). The remaining signals belonged to one chelated hydroxyl proton (δ_H 12.75) and six protons (δ_H 4.03, 4.11) corresponding to two methoxyl group. The NMR spectra data indicated the tetrasubstituted skeleton for this compound. Correlations between the chelated hydroxyl proton with C-1, C-2 and C-9a confirmed the position C-1 of this proton. The position of other substituted groups were also deduced from the correlations in the HMBC spectrum. By full analyses of 1D and 2D NMR spectra, the structure of **3** was identified as 1,6-dihydroxy-5,7-dimethoxyxanthone. This compound have been first isolated from *Hypericum ascyron* and *Caraipa densiflora* [7].

Table 1. ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data of **1, 2, 3.**

	1		**2**		**3**	
	δ_C (ppm)	δ_H (m, Hz)	δ_C (ppm)	δ_H (m, Hz)	δ_C (ppm)	δ_H (m, Hz)
1	154.46	-	161.26	-	161.76	-
2	131.75	-	109.63	6.71 (dd, 8.5, 0.5)	110.47	6.80 (dd, 8.5, 0.5)
3	159.90	-	136.56	7.52 (t, 8.5)	136.10	7.57 (t, 8.5)
4	89.89	6,42 (s)	107.09	6.87 (dd, 8.5, 0.5)	110.47	7.0 (dd, 8.5, 0.5)
4a	152.96	-	156.40	-	156.11	-

4b	150.76	-	150.24	-	146.34	-
5	113.87	7,16 (d, 9.0)	119.07	7.33 (d, 9.0)	145.01	-
6	122.63	7.38 (d, 9.0)	125.37	7.25 (dd, 9.0, 3.0)	145.77	-
7	145.57		153.65	-	134.65	-
8	144.25		108.20	7.47 (d, 2.5)	99.79	7.44 (s)
8a	114.51	-	120.77	-	113.20	-
9	180.90	-	182.41	-	181.19	-
9a	104.45	-	108.46	-	108.53	-
2-OCH_3	60.91	3.91 (s)	-	-	-	-
3-OCH_3	56.31	3.96 (s)	-	-	-	-
8-OCH_3	62.84	4.03 (s)	-	-	-	-
1-OH		13.02 (s)	-	12.60 (s)	-	12.75
5-OH		6.00 (s)	-	-	-	
7-OH				3.83 (s)	-	-
5-OCH_3					56.59	4.03 (s)
7-OCH_3					61.73	4.11 (s)

4. CONCLUSION

In continuation of our investigation on the phytochemistry of the Vietnamese *Calophyllum* species. Three xanthones were isolated from the dichloromethane extract of *Calophyllum ceriferum* Gagnep. Ex Stevens collected at Ninh Thuan province. Their structure were identified as 1,7-dihydroxy-2,3,8-trimethoxyxanthone, 1,7-dihydroxyxanthone and 1,6-dihydroxy-5,7-dimethoxyxanthone by means of spectroscopic methods. These compounds are being evaluated for the antioxidant and antibacterial activities, and cytotoxicity on some human cancer cell lines.

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TÓM TẮT

CÁC HỢP CHẤT XANTON TỪ CÀNH NHỎ CÂY CÒNG SÁP (*CALOPHYLLUM CERIFERUM* GAGNEP. EX STEVEN) Ở VIỆT NAM

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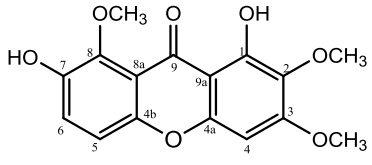
Chi Còng (*Calophyllum*) là một trong số các chi thuộc họ Măng cụt (Clusiaceae). Ở Việt nam, chi này có 15 loài nhưng còn ít được nghiên cứu về thành phần hóa học cũng như hoạt tính sinh học. Các nghiên cứu trước đây của chúng tôi về 2 loài Mù u (*C. inophyllum*) và Rù ri (*C. balansae*) của Việt Nam cho thấy chúng là nguồn giàu có các hợp chất coumarin, tecpenoit và xanton. Tiếp tục hướng nghiên cứu này, chúng tôi đã phân lập và xác định được cấu trúc của 3 hợp chất xanton là 1,7-dihydroxy-2,3,8-trimethoxyxanthone (**1**), 1,7-dihydroxyxanthone (**2**) and 1,6-dihydroxy-5,7-dimethoxyxanthone (**3**) từ dịch chiết diclometan của lá cây Còng sáp (*C. ceriferum*) mọc ở tỉnh Ninh Thuận. Các hợp chất này đang tiếp tục được đánh giá về một số hoạt tính sinh học như kháng vi sinh vật kiểm định, chống ôxi hóa và khả năng gây độc tế bào trên một số dòng tế bào ung thư người.



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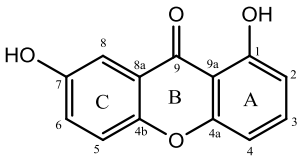

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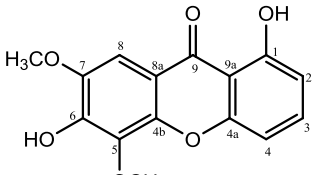

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Từ khóa: còng sáp, xanton, 1,7-dihydroxy-2,3,8-trimethoxyxanthone, 1,7-dihydroxyxanthone, 1,6-dihydroxy-5,7-dimethoxyxanthone, Ninh Thuận.