CHEMICAL CONSTITUENTS ISOLATED BY ACETYLATION OF *ERIOBOTRYA ANGUSTISSIMA*'S LEAVES

CÁC HỢP CHẤT HÓA HỌC ĐƯỢC PHÂN LẬP TỪ DẪN XUẤT ACETYL HÓA CỦA LÁ CÂY SƠN TRÀ HẸP (*ERIBOTRYA ANGUSTISSIMA* HOOK.F.)

Giang Thi Kim Lien

The University of Danang; Email: giangkimlien@gmail.com

Abstract - Eriobotrya angustissima Hook.F. (Son tra hep) belongs to the family Rosaceae. Its leaves were collected at National Garden Bi Dup in Lam dong province. The research showed that, many triterpenes are very difficult to be separated from each other so that they can be obtained only as acetylated products. From a fraction of the ethyl acetate extract from Eriobotrya angustissima's leaves, four acetylated products (1a) 3β-O-acetylursolic acid, (2a) 3β-O-acetylpomolic acid, (3a) 2α,3α-di-O-acetyl-19α-hydroxy-12-ursene-28-oic acid and (3b) 2α-O-acetyl-3α,19α-dihydroxy-12-ursene-28-oic acid were isolated from this fraction. The structure of these compounds was determined using MS, NMR spectroscopic data and by comparison with reported data. This is the first report on chemical constituents of Eriobotrya angustissima growing in Vietnam.

Key words - *Eriobotrya; Eriobotrya angustissima* Hook.F; acetylation; triterpenes; ursolic acid.

Tóm tắt - Cây Sơn trà hẹp (*Eriobotrya angustissima* Hook. f.) thuộc họ Hoa hồng được thu hái tại Vườn quốc gia Bì Đúp, Lâm Đồng. Nhóm nghiên cứu của chúng tôi đã phân lập và xác định được cấu trúc của một số hợp chất hóa học từ dịch chiết lá cây Sơn trà. Trong số các chất này, có những hợp chất không thể phân lập trực tiếp từ dịch chiết mà phải phân lập gián tiếp qua dẫn xuất acetyl hóa của nó. Bài báo này trình bày kết quả phân lập các hợp chất hóa học từ dịch chiết của lá cây Sơn trà hẹp bằng dẫn xuất acetyl hóa. Cấu trúc hóa học của chúng được xác định là (**1a)** 3β-O-acetylursolic acid; (**2a)** 3β-O-acetylpomolic acid; (**3a**) 2α,3α-di-O-acetyl-19α-hydroxy-12-ursene-28-oic acid và (**3b**) 2α-O-acetyl-3α,19α-dihydroxy-12-ursene-28-oic acid bằng việc kết hợp các phương pháp phổ khối lượng MS, phổ cộng hưởng từ hạt nhân NMR. Đây là những kết quả nghiên cứu bước đầu về thành phần hóa học của cây Sơn trà hẹp ở Việt Nam.

Từ khóa - Chi Sơn trà; Cây Sơn trà hẹp; acetyl hóa; triterpene; ursolic acid.

1. Introduction

In Vietnam, the genus *Eriobotrya* (Rosaceae) consists of 13 species, originated from East and South East Asia [1]. Only one of these species, the edible and medicinally used *Eriobotrya japonica* (Thun.) Lindl. (loquat) has been hitherto intensively studied and has showed several interesting chemical constituents and biological activities, for example triterpenoids with anti-oxidant activity, polyphenols and megastigmane glycosides with antitumor effects [2 - 4]. These results prompted us to further investigate a unstudied species growing in Viet nam: *E. angustissima* Hook. f. This *Eriobotrya* plant can be found in high mountain areas of Vietnam.

The leaves of *E. angustissima* were collected in March 2009 at the Bi Dup national park, Nui Ba, Lam Dong province of Vietnam and evaluated by Dr. Nguyen Tien Hiep, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

Four components were determined: (**1a)** 3β-O-acetylursolic acid; (**2a)** 3β-O-acetylpomolic acid; 2α,3α-di-O-acetyl-19α-hydroxy-12-ursene-28-oic acid (**3a**) and 2α-O-acetyl-3α,19α-dihydroxy-12-ursene-28-oic acid (**3b**) from an acetylated fraction.

2. Experimental

2.1. Instruments and Chemicals

NMR: Varian Unity 300; MS: AMD 402; For analytical purposes: Merck TLC aluminium sheets silica gel 60 F_{254} (layer thickness 0.2 mm) were used. Silica gel Merck 60 (0.040 - 0.063 mm) is used for column chromatography.

2.2. Acetylaction and Isolation method

The leaves of *E. angustissima* (0.5 kg) were treated and extracted with the same procedure as for *E. poilanei.* The ethyl acetate extract (19.0 g) was chromatographed over silica gel column, and gradiently eluted with n-hexane/ethyl acetate and then with ethyl acetate/methanol to obtain 20 fractions.

Acetylation: to increase the ability to separate the compounds, 15th and 16th fractions are combined and implemented acetylation reaction with 3 ml of acetic anhydride and 1 ml of pyridine at room temperature for about 24 hours. After the reaction mixture was added with water, and then extracted several times with CH_2Cl_2. The gross extracts CH_2Cl_2 was washed with water, distilled solvents under reduced pressure. 950 mg of product mix was collected from acetylation.

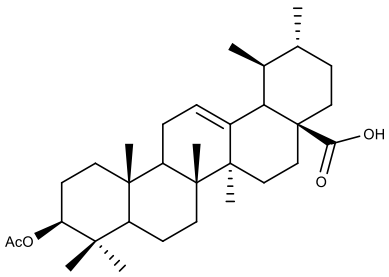
The acetylated product was chromatographed by using silica gel column to give 4 triterpene acids, including 3β-*O*-acetylursolic acid **(1a)**, 3β-*O*-acetylpomolic acid **(2a)**, 2α,3α-*O*-diacetyl-19α-hydroxy-12-ursene-28-oic acid (**3a**, ESI-MS: $[M+Na]^+$, *m/z* 595) and 2α-*O*-acetyl-3α,19α- dihydroxy-12-ursen-28-oic acid (**3b**, ESI-MS: $[M+Na]^+$, *m/z* 553).

3. Results and discussion

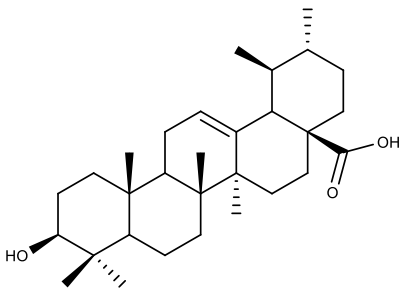
Four components were identified as (**1a)** 3β-O-acetylursolic acid; (**2a)** 3β-O-acetylpomolic acid; 2α,3α-di-O-acetyl-19α-hydroxy-12-ursene-28-oic acid (**3a**) and 2α-O-acetyl-3α,19α-dihydroxy-12-ursene-28-oic acid (**3b**) from an acetylated fraction.

The compounds (**1a**) and (**2a**) were identified as ursolic acid 3β-acetate, pomolic acid 3β-acetate respectively by comparing them with standard compounds. Thus, their original compounds were ursolic acid (**1**) and pomolic acid (**2**). These standards were isolated from *Eriobotrya*

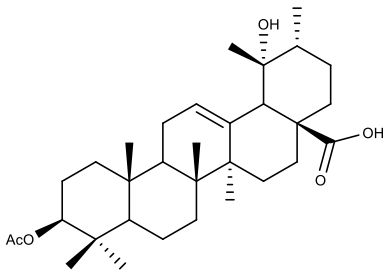
poilanei and their structure was determined by our research group [5, 6, 7].



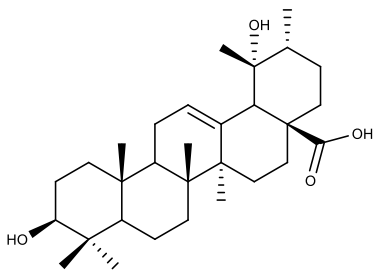
***(1a)**: Ursolic acid 3β-acetate*



***(1)**: Ursolic acid*



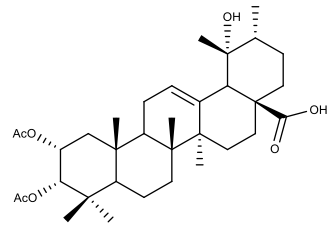
***(2a)**: Pomolic acid 3β-acetate*



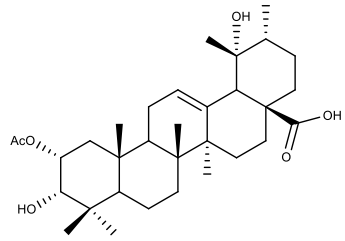
***(2)**:Pomolic acid*

The substance (**3a**) was obtained as white powder. It showed the characteristic absorption bands at 3445 cm^{-1} (OH) and 1737 cm^{-1} (CO) in the IR spectrum. The ESI – MS spectrum showed the pseudo molecular ion peak at m/z=595$[M+Na]^+$. The ^{13}C-NMR spectrum indicated 34 carbons in the molecule of (**3a**). Data from the DEPT and MS spectra suggested that the molecular formula of (**3a**) is $C_{34}H_{52}O_7$. The ^{13}C-NMR-DEPT spectrum showed that in addition to the 4 signals of the 2 acetyl group, the remaining 30 carbon atoms include one carboxyl group (δC=183.24), 8 methylene groups (CH2), 7 methin groups (CH), 7 quaternary carbons, in which two methin groups and 1 quaternary carbon have oxygen (δC=77.16; 73.13 and 68.25) and 7 methyl groups attached to them. The ^{1}H-NMR spectrum pointed out the presence of a double bond type >C=CH- [δH=5.35 (1H,t,J=3.5); δC=138.02 s, 129.06 d], and 7 methyl groups ranging from δH=0.74 to 1.29 ppm including 6 quaternary methyl groups at δH = 0.74, 0.88, 0.98, 1.04, 1.21, 1.29 and 1 ternary methyl groups represented by a doublet signal at δH=0.95 (3H, d, J=6.5 Hz). The spectral data analysis above allows prediction for EAE - AC2 to be a 12-ursane triterpen acid skeleton containing 1 ternary hydroxy group, and 2 acetyl groups.

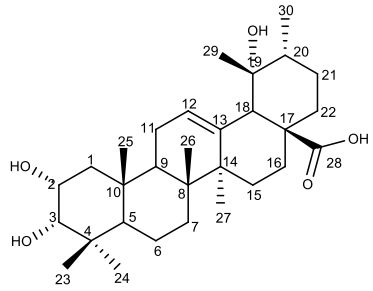
The chemical shifts of two acetoxy methin protons at δH=5.22 (1H, ddd, J = 12.5, 4.0, 3.0) and δH = 4.97 (1H, d, J = 3.0) were assigned to H-2 and H-3 in ring A of the molecule in comparison with document [6]. The small interaction constant J=3.0 Hz between H-2 and H-3 showed that they are on the same side of the molecular plane. In addition, the signal of the quaternary carbon at δC = 73.13 indicated that there was a hydroxyl group connected to C-19 in the molecule. Consequently, the structure of (**3a**) was determined to be 2α,3α-di-O-acetyl,19α-hydroxy-urs-12-en-28-oic acid by comparing with the published data [8].



***(3a):** 2α,3α-di-O-acetyl,19α-hydroxy-urs-12-en-28-oic acid*



***(3b):** 2α-O-acetyl,3α,19α-dihydroxy-12-ursen-28-oic acid*



***(3)**: 2α,3α,19α- trihydroxy-12-ursen-28-oic acid.*

(**3b**)'s ^{1}H and ^{13}C-NMR spectra were similar to those of (**3a**) except that there was in only one acetyl group in the (**3b**) molecule at [δH = 2.07, δC = 21, 30, 170.17] and the resonance signal of H-3 at δH = 3.49 (1H, d, J=2.5) on the 1H - NMR spectrum was shifted toward the higher field than H-3 of the (**3a**) molecule (ΔδH = 1.48 ppm). This demonstrates that the hydroxy group is attached at C-3.

Thus, the structure of (**3b**) was identified as 2α-O-acetyl,3α,19α-dihydroxy-12-ursen-28-oic acid. Through comparison between (**3a**) and (**3b**) and their fraction before acetylation, the origin substance in the ethyl acetate extract from the *Eriobotrya angustissima* Hook. f. was determined as 2α,3α,19α-trihydroxy-12-ursen-28-oic acid (substance **3**). This substance is also called euscaphic acid, whose methylated product, methyl euscaphate, had been previously isolated from *Euscaphis japonica* and *Isodon arboreus* [8].

Table 1. *13C-NMR spectral data of (**3a**), (**3b**) and Methyl euscaphate (125 MHz, CDCl3)*

C	(**3a**)	(**3b**)	Methyl euscaphate [8]
1	38,83 t	37,92 t	38,7 t
2	68,25 d	71,17 d	68,2 d
3	77,16 d	76,87 d	77,1 d
4	38,45 s	38,51 s	38,3 s
5	49,58 d	48,01 d	49,5 d
6	18,22 t	18,02 t	17,9 t
7	32,46 t	32,52 t	32,4 t
8	40,16 s	40,19 s	40,0 s
9	47,73 s	47,74 s	47,0 s
10	38,13 s	38,50 s	38,1 s
11	23,66 t	23,70 t	23,6 t
12	129,05 d	129,14 d	128,8 d
13	138,02 s	138,02 s	138,1 s
14	41,24 s	41,26 s	41,2 s
15	28,19 t	28,16 t	28,1 t
16	25,36 t	25,38 t	25,4 t
17	47,73 s	47,74 s	47,8 s
18	52,85 d	52,89 d	53,1 d
19	73,13 s	73,10 s	73,1 s
20	41,09 d	41,09 d	41,1 d
21	25,99 t	25,99 t	25,9 t
22	37,46 t	37,51 t	37,3 t
23	27,74 q	28,33 q	27,7 q
24	21,56 q	21,85 q	21,5 q
25	16,12 q	16,19 q	16,1 q
26	17,04 q	17,05 q	16,6 q
27	24,6 q	24,66 q	24,6 q
28	183,01 s	183,24 s	178,3 s
29	27,39 q	27,39 q	27,3 q
30	16,12 q	16,14 q	16,1 q
Ac	170,64 s	-	170,7 s
Ac	170,43 s	170,18 s	170,4 s
	20,98 q	-	21,1 q
	21,09 q	21,38 q	21,1 q

4. Result

From a fraction of the ethyl acetate extract from *Eriobotrya angustissima's* leaves, four acetylated products were isolated. The structure of these compounds was determined using MS, NMR spectroscopic data and by comparison with reported data, that were 3β-O-acetylursolic acid (**1a**), 3β-O-acetylpomolic acid (**2a**), 2α,3α-di-O-acetyl-19α-hydroxy-12-ursene-28-oic acid (**3a**) and 2α-O-acetyl-3α,19α-dihydroxy-12-ursene-28-oic acid (**3b**). Through comparison between (**3a**) and (**3b**) and their fraction before acetylation, the origin substance in the ethyl acetat extract from the *Eriobotrya angustissima* Hook. f. was determined as 2α,3α,19α-trihydroxy-12-ursen-28-oic acid.

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