Malaysian Journal of Science (**24**): 149-153 (2005)

Pyranocoumarins from the roots of *Acronychia pedunculata*

N. Md. Ali, J. Latip and ***E. Ismail**

School of Chemical Sciences and Food Technology, *School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia 43600, Bangi

Abstract. Phytochemical investigation on the roots of *Acronychia pedunculata* (mentua Keminiyan) has been conducted. Several chromatographic methods have been employed to the ethyl acetate extract of the roots which led to the isolation of two pyranocoumarins namely alloxanthoxyletin and xanthoxyletin. Their structures were determined by extensive physical studies particularly NMR. The petroleum ether, ethyl acetate and ethanol extracts and the pure compounds of *Acronychia pedunculata* were tested for their cell growth inhibitory property against MCF-7 cell lines. The results showed that only the ethyl acetate extract was found to be moderately toxic with IC_{50} values of 93μg/ml.

Keywords : Rutaceae, *Acronychia pedunculata*, pyranocoumarins, NMR, cytotoxicity

Abstrak. Kajian fitokimia telah dijalankan ke atas akar *Acronychia pedunculata* (Mentua Keminiyan). Pelbagai kaedah kromatografi telah dilakukan ke atas ekstrak kasar etil asetat akar dan berjaya mengasingkan dua sebatian piranokumarin iaitu alloxantoxailetin dan xantoxailetin. Pencirian struktur kedua-dua sebatian ini dilakukan berdasarkan analisis fizikal terutamanya RMN. Ekstrak kasar petroleum eter, etil asetat dan etanol serta komponen tulen telah diuji kesan perencatannya ke atas pertumbuhan sel tumor MCF-7. Keputusan mendapati hanya ekstrak kasar etil asetat sahaja yang menunjukkan kesitotoksikan yang sederhana dengan nilai IC_{50}, 93μg/ml.

INTRODUCTION

Acronychia pedunculata (Mentua Keminiyan) from the family of Rutaceae is a moderate size tree (30 m) which distributed throughout Peninsular Malaysia. This species is *synonym* to *Acronychia laurifolia, A. arborea*, and *A. resinosa*; which can be found in China, India, Indo-China and Indonesia [1, 2]. This plant has been used traditionally to treat skin problems, stomachic and as a pain killer, while its roots are used to produce charcoal [2]. Previous studies on this plant have been reported to contain alkaloids [3-5], coumarins [6], chromenes [4], triterpenes [4, 6, 7], acetophenones derivatives [4, 8-11] and lignans [5]. In this paper, we report the isolation and characterisation of two pyranocoumarins from the ethyl acetate extract of the roots as well as the cytotoxicity activity of the crude extracts and pure compounds.

EXPERIMENTAL METHOD

Melting point was measured on Gallenkamp and was uncorrected. UV spectra was recorded on Shimadzu UV-160 while IR spectra was obtained with $CHCl_3$ as the solvent on Perkin Elmer FTIR 1725-X spectrometer. ^1H (400 MHz), ^{13}C (100.56 MHz) and 2D-NMR measurements were carried out on JEOL ECP-400 spectrometer. Chemical shifts are reported in ppm and the coupling constants are given in Hz. Gas chromatography-mass spectra were obtained on a Macromass LCT.

Extraction and isolation

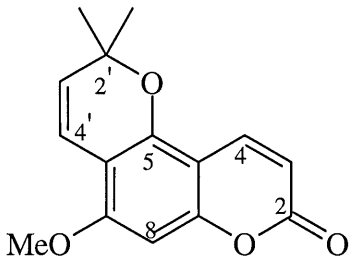
The roots of *Acronychia pedunculata* were collected from Endau-Rompin Forest Reserve, Pahang and a voucher specimen was deposited at UKMB herbarium of Malaysia. The roots were air dried, ground and successively extracted with Soxhlet apparatus in petroleum ether, ethyl acetate and ethanol to yield the petroleum ether, ethyl acetate and ethanol extracts. The ethyl acetate extract was subjected to Vacuum Liquid Chromatography (VLC) over silica gel 60H using the mixtures of

hexane/ethyl acetate and ethyl acetate/ethanol to afford 20 fractions (FG1-FG20). Fraction FG7 obtained from VLC eluted with hexane/ethyl acetate (4:6) was further purified over Sephadex LH-20 column eluted with mixtures of hexane/chloroform (8:2) to afford alloxanthoxyletin ***1*** (13 mg). Similar procedure was done on FG8 obtained from VLC eluted with mixture of hexane/ethyl acetate (3:7) to give xanthoxyletin ***2*** (21 mg).

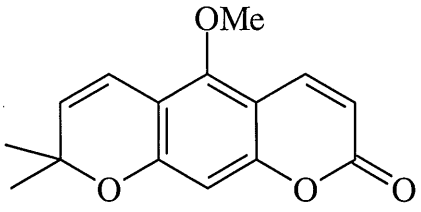
Cytotoxicity assay

MCF-7 human breast carcinoma cell lines were cultured as recommended. The cytotoxicity assay was done based on Ali *et al.* [12] with slight modification. 200µl of medium containing the minimum of 5 x 10^5cell/ml was added to each well of the 96-well microtiter plate. After 24 hours, each well was added with 2µl of extracts/pure compounds at different concentrations, while control well only contained DMSO solution. After 24 hours incubation (37^0C, 10% CO_2), 20µl of MTS was added to each well and the plates were incubated for another 4 hours to enable viable cells to metabolise the soluble tetrazolium salts to formazan. The absorbance of formazan solution (in DMSO) as a measure of viable cell number was determined at 570nm using ELISA Dynatech MR5000 plate reader. The IC_{50} values (concentration of tested compound required to inhibit cell proliferation by 50%) were determined from dose-response inhibition curves.

RESULTS AND DISCUSSION



Alloxanthoxyletin, ***1***



Xanthoxyletin, ***2***

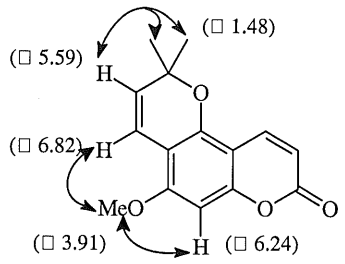
Compound ***1*** was obtained as pale yellow needles crystal (m. p. 115^0C), which exhibited an M^+ at *m/z* 258, indicating the molecular formula of $C_{15}H_{14}O_4$. In addition, the base peak appeared at *m/z* 243 (M^+ – 15) corresponded to the loss of methyl from the molecular ion. The UV absorption bands at λ_{max} 221, 281 and 315 nm were consistent to the benzopyrone chromophore [13]. The IR spectrum of ***1*** showed strong absorption bands at 1658 (C=O) and 1600cm^{-1} (C=C aromatic), which further support the benzopyrone nature [13].

The ^{1}H NMR (Table 1) of ***1*** displayed 4 doublets, at δ 6.15 and δ 7.98 (each J = 9.88Hz) were attributable to H-3 and H-4 of the pyrone ring respectively, and at δ 6.82 and δ 5.59 (each J = 10 Hz) were typical of olefinic protons in a pyran system [14]. The presence of pyran ring in structure ***1*** was further supported by an upfield singlet at δ 1.48 (6H) indicated the typical *gem*-dimethyls substituted pyran ring [14]. The remaining signals are two singlets at δ 6.24 and δ 3.91, consistent to the presence of one aromatic proton and a methoxyl substituent. The ^{13}C NMR spectrum (Table 1) of ***1*** exhibited 14 carbon resonances, which suited the methoxyl substituted 2, 2-dimethylpyranocoumarin structure [14]. All C-H carbons were correctly assigned based on the ^{1}H-^{13}C direct correlations observed in the HMQC spectrum of ***1***.

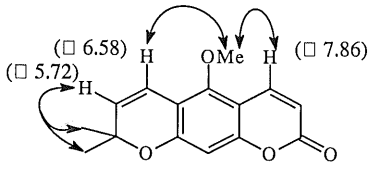
The position of the methoxyl (δ 3.91) and aromatic singlet (δ 6.24), as well as the olefinic protons were confirmed by means of the NOESY and HMBC experiment. Dipolar interaction was observed in the NOESY spectrum of ***1*** (Figure 1a), between the methoxyl signal (δ 3.91) and aromatic singlet (δ 6.24), in which this methoxyl showed further interaction with the olefinic proton at δ 6.82. Both interactions required the placement of methoxyl and aromatic singlet at C-7 and C-8 respectively and a doublet at δ 6.82 (H-4), thus ***1*** has to be an angular pyranocoumarin. Furthermore, the NOESY interaction was also observed between the *gem*-dimethyls (δ 1.48) and a doublet at δ 5.59, which confirmed their placement at C-2' and C-3' of the pyran ring respectively.

The placement of all protons and quaternary carbons were confirmed by the ^{1}H-^{13}C long-range correlations observed in the HMBC

spectrum of ***1*** (Figure 2a). Based on its spectral data and comparison with those of literature [13, 14], compound ***1*** was identified as 7-methoxy-2', 2'-dimethylpyranocoumarin namely alloxanthoxyletin.



(a)



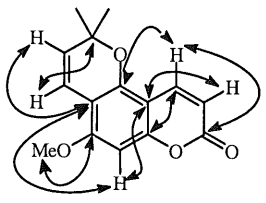
(b)

Figure 1. Dipolar interactions observed in NOESY spectra ***1*** (a) and ***2*** (b)

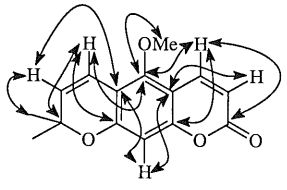
Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) data of 1 and 2

	1		**2**	
Carbon	δ_H	δ_C	δ_H	δ_C
2	-	161.6	-	161.2
3	6.15, d (9.88)	110.7	6.22, d (9.88)	112.5
4	7.98, d (9.88)	139.2	7.86, d (9.88)	138.7
4a	-	103.9	-	111.5
5	-	151.3	-	153.0
6	-	102.8	-	107.6
7	-	157.6	-	155.8
8	6.24, s	95.6	6.56, s	101.0
8a	-	156.7	-	157.7
2'	-	77.5	-	76.9
3'	5.59, d (10.0)	127.8	5.72, d (10.0)	130.8
4'	6.82, d (10.0)	115.2	6.58, d (10.0)	116.0
OCH_3	3.91, s	56.2	3.86, s	63.9
Gem-CH_3	1.48, s	28.4	1.46, s	28.3

Coupling constant, *J* in parentheses



(a)



(b)

Figure 2. Selected ^{3}J interactions observed in HMBC spectra of ***1*** (a) and ***2*** (b)

Compound **2** was crystallized from hexane as fine yellow needles, m.p. 131 – 132^{0}C. The UV and IR spectral data of *2* were resembled those of *1*, suggested that *2* was also a pyranocoumarin. The mass spectrum of *2* gave an M^+ at *m/z* 258, consistent to the molecular formula of $C_{15}H_{14}O_4$. The ^{1}H NMR spectrum showed almost identical pattern to that of pyranocoumarin *1*, with four doublets at δ 6.22 (*J* = 9.88 z, H-3), δ 7.86 (*J* = 9.88 Hz, H-4), δ 5.72 (*J* = 10 Hz, H-3') and δ 6.58 (*J* = 10 Hz, H-4'); one aromatic singlet at δ 6.56, one methoxyl singlet at δ 3.86 and an upfield *gem*-dimethyls singlet at δ 1.46.

The ^{13}C NMR spectrum of *2* was typical of the methoxyl substituted pyranocoumarin skeleton [13, 15], with methoxyl carbon resonated further downfield at δ 63.9, as compared to *1*. Panichpol & Waterman reported that the downfield shift of methoxyl signal was due to the steric hindrance between the *O* of the OMe and the aromatic ring in which, the deshieding effect is seen only when the OMe is *diortho* substituted by two bulky substituents such as OMe, or OH, or a ring junction [16]. Furthermore, the resonance of H-4 was also relatively deshielded with a long range coupling (W-coupling) observed in ^{1}H NMR and ^{1}H-^{1}H COSY spectra of *2*, indicated the presence of an oxygen substituent at C-5 position and C-8 is unsubstituted [13, 15]. Both observations suggested that 2, 2-dimethylpyran ring was fused at different positions of coumarin skeleton as compared to *1*.

The complete structure of *2* was determined by means of the NOESY and HMBC experiments. Interactions through space were observed between the methoxy signal (δ 3.86) with H-4 (δ7.86) and H-4' (δ6.58) in the NOESY spectrum of *2*, requiring the placement of methoxyl group at C-5, thus the 2, 2-dimethylpyran ring is linearly located to the coumarin structure. Such arrangements in structure *2* were further confirmed by long-range ^{1}H-^{13}C interactions observed in the HMBC spectrum (Figure 2b). Pyranocoumarin *2* was identified as 5-methoxy, 2', 2'-dimethylpyranocoumarin, which is commonly known as xanthoxyletin based on its physical properties and comparison with literature data [13, 15]. Pyranocoumarins known as sesaline and norbrayiline have been reported from *A. laurifolia* [6].

The cell growth inhibitory property of petroleum ether, ethyl acetate and ethanol extracts, alloxanthoxyletin and xanthoxyletin were evaluated based on MTS assay. Of all the three crude extract's tested, only the ethyl acetate extract showed some cell growth inhibitory activity (IC_{50} = 93 μg/ml) when tested using human breast carcinoma cells, MCF-7. However, both of the ethyl acetate's derived pure compounds, alloxanthoxyletin and xanthoxyletin were not active. This might be due to synergistic requirement of these pure compounds by which the presence of more than one compounds are needed to produce some cell growth inhibitory action unlike estrogen receptor inhibitor, tamoxifen which solely gave an IC_{50} value of 19.2 μg/ml.

REFERENCES

1. Ridley, H. N., 1967, *The Flora of The Malay Peninsular*. L. Reeve Ang Co.Ltd.
2. Burkill, I. H. 1966. A Dictionary of the Economic Products of Malaysia Peninsula. 2nd ed. Kuala Lumpur :112.
3. De Silva, L. B., De Silva, U. L. L., Mahendaran, M. and Jennings, R. 1979. *Phytochemistry*. **18** : 1255.
4. Kumar, V., Karunaratne, V. and Meegalle, M. R. S. K. 1989. *Phytochemistry*. **28** : 1278.
5. Cui, B., Chai, H., Dong, Y., Horgen, F. D., Hansen, B., Madulid, D. A., Soejarto, D. D., Farnsworth, N. R., Cordell, G. A., Pezzutto, J. M. and Kinghorn, D A.. 1999. *Phytochemistry*. **52** : 95.
6. Rahmani, M., Taufik, Y. Y. H. and Sukari, M. A. 1996. *Fitoterapia*. **67** : 10.
7. Lahey, F. N. and Leeding, M. V. 1958. *Proc. Chem. Soc*. **73** : 42.
8. Biswas, G. K., Chattajee, A. 1970. *Chemistry & Industry*. **20** : 654.
9. Baneerji, J. R. N. and Chattajee, A. 1973. *Indian J. Chem*. **11** : 693.
10. De Silva, L. B., Herath, W. M., Liyanage, C., Kumar, V., Ahmad, V. U. and Sultana, A. 1991. *Phytochemistry*. **30** : 1709.
11. Sy, L. K. and Brown, G. D. 1999. *Phytochemistry*. **52** : 681.
12. Ali, A. M., MacKeen, M. M., Hamid, M., Aun, Q. B., Zauyah, Y., Azimatol,

H. L. P. and Kawazu, K. 1997. *Planta Medica.* **63** : 81.

13. Murray, R. D., Mendez, J. S. & Brown, S. A. 1982. *The Natural Coumarins, Occurrence, Chemistry & Biochemistry*. New York : John Wiley & Sons Ltd.

14. Ganguly, A. K., Joshi, B.S., Kamat, V. N. & Manmade, A. H., 1967. *Tetrahedron*. **23** : 4777.

15. Kumar, V., Mohamed, N. N. M., Saminathan, S. and Wickramaratne, D. B. M. 1998. *Phytochemistry*. **49** : 215.

16. Panichpol, K. and Waterman, P. G. 1978. *Phytochemistry*. **17** : 1363.