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Cuneifolin, a New Xanthone from *Garcinia cuneifolia* (Guttiferae)

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Abstract – Studies on the stem bark extracts of *Garcinia cuneifolia* have furnished a new xanthone cuneifolin (**1**) and the triterpene stigmasterol (**2**). Structures for these compounds were elucidated based on NMR, 2D NMR, MS and GCMS data. Larvicidal activity screening of the crude bark extract using the larvae of *Aedes aegypti* indicated the larvae to be susceptible to these extracts. LC_{50} values of the bioassays show the extracts to be moderately toxic to the larvae of *Aedes aegypti*.
Keywords – *Garcinia cuneifolia*, xanthone, larvicidal, *Aedes aegypti*, toxicity

Introduction

Members of the Guttiferae are known to be a rich source of xanthones (Bennet G.J. and Lee H.H., 1989; Sultanbawa M.U.S., 1980). Prenylated xanthones commonly found in *Garcina* have been reported to show antibacterial and antifungal activities (Iinuma M. *et al.*, 1996). The genus *Garcinia*, also a member of the Guttiferae family have been used in Thai folk medicine for its antipyretic property. This genus is rich in prenylated xanthones (Xu Y-J *et al.*, 2000) triterpenes (Chung M-I *et al.*, 1998), biflavonoids (Lin Y-M *et al.*, 1997) and polyprenylated benzophenones (Roux D. *et al.*, 2000) which are biologically active (Iinuma M. *et al.*, 1996). Extensive research has shown that *Garcinia* species exhibited a wide range of biological and pharmacological activities such as cytotoxic, antimicrobial, antimalarial and anti-HIV-1 protease inhibitory activity (Kosela S. *et al.*, 2000).

Experimental

General – ^1H and ^{13}C NMR spectra were recorded on a JEOL FTNMR 400MHz spectrophotometer using $CDCl_3$ as solvent and TMS as the internal standard. EIMS was recorded on a Shimadzu GCMS QP5000 instrument equipped with a direct injection probe.

Plant material – The stem bark of *Garcinia cuneifolia* was collected from the Sri Aman district in Sarawak, East Malaysia. Identification of the plant specimen was carried out at the Forest Research Centre, Kuching, Sarawak, Malaysia.

Extraction and Isolation – The finely ground stem bark of *Garcinia cuneifolia* (1 kg) was extracted with n-hexane twice to yield 5.4 g of crude extract. The dry crude extract was purified by filtering column chromatography. Further purifications were carried out using preparative thin layer chromatography or recrystallisations. This gave the new xanthone cuneifolin (100 mg) and the common triterpene stigmasterol (150 mg). The xanthone was identified using ^1H, ^{13}C NMR, 2D NMR and Mass spectrometry.

Compound 1 (Cuneifolin): Yellow oily powder; IR (KBr) cm^{-1}: 1720,1645,1583; EIMS: m/z 478 (M^+); HRCIMS (methane): m/z 479.24106 (calcd 479.24342); ^1H NMR ($CDCl_3$, 400 MHz) δH (ppm): 13.47 (1H, s, 1-OH), 7.04 (1H, brs, 4-OH), 6.59 (1H, brs, 6-OH), 6.77 (1H, s, H-7), 6.34 (1H, s, H-2), 3.89 (3H, s, 3-OMe), 5.21 (1H, brt , J = 6.1 Hz, H-12), 3.48 (1H, brd, J = 6.3 Hz, H-11), 2.01 (1H, m, H-14), 1.75 (1H, s, H-15), 2.06 (1H, m, H-16), 5.04 1H, brt, J = 6.7 Hz, H-17), 1.60 (1H, s, H-19), 1.55 (1H, s, H-20), 3.95 (1H, brd, J = 7.0 Hz, H-21), 5.35 (1H, brt, J = 7.0 Hz, H-22), 1.72 (1H, s, H-24), 1.83 (1H, s, H-25). ^{13}C NMR ($CDCl_3$, 100 MHz) δC (ppm): 163.2 (C-1), 94.3 (C-2), 162.3 (C-3), 56.0 (3-OMe), 148.1 (C-4), 106.5 (C-5), 146.2 (C-6), 113.2 (C-7), 128.7 (C-8), 182.5 (C-9), 137.5 (C-4a), 111.6 (C-8a), 103.4 (C-9a), 153.0 (C-10a), 21.6 (C-11), 123.0 (C-12), 135.3 (C-13), 39.5 (C-14), 17.9 (C-15), 26.6 (C-16), 124.0 (C-17), 133.2 (C-18), 25.9 (C-19), 17.7 (C-20), 32.9 (C-21), 122.4 (C-22), 131.6 (C-23), 25.6 (C-24), 16.2 (C-25).

Toxicity assay – Bioassays for the toxicity of the crude extracts were carried out against mosquito larvae of *Ae.*

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Aegypti using the WHO standard procedures with slight modifications (World Health Organization, 1980). A stock solution was prepared by dissolving a weighed amount of the crude extract in absolute ethanol. Serial dilutions of the stock were prepared in clean 250 ml drinking glasses containing 50 ml of test solution and 10 late third instar mosquito larvae. A small amount of larval food was added. After twenty-four hours, mortality counts were made. Experiments were carried out in duplicates or triplicates. The mortality data were analyzed with a personal computer programmed with probit analysis in order to obtain LC_{50} and LC_{90} values.

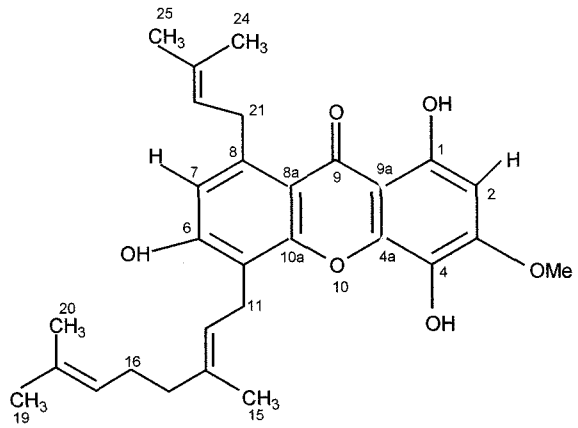

Fig. 1. Structure of Cuneifolin.

Results and Discussion

The hexane extract of the stem bark of *Garcinia cuneifolia* furnished two compounds one of which is a new compound while the other the common triterpene stigmasterol. The structure of the new compound cuneifolin was elucidated using ^{1}H, ^{13}C, 2D NMR and MS techniques.

Cuneifolin was isolated as a yellow oily powder. The EIMS spectrum gave an M^+ of 478 indicating a molecular formula of $C_{29}H_{34}O_6$. HRCIMS (methane): 479.24106 (calcd 479.24342). This compound gave IR absorption bands at 1720, 1645 and 1583 while the UV spectrum gave a maximum absorption at 324 nm. The ^{1}H NMR spectrum showed the presence of one chelated hydroxyl group at δ13.50 and a methoxy group at δ3.89. Two one proton singlets were observed at δ 6.34 and δ6.77 indicating the presence of only two non coupled hydrogens in the xanthone rings. Five three-hydrogen methyl singlets at δ1.55, 1.60, 1.72, 1.75 and 1.83 and four methylene CH_2 signals at δ3.48 (d, J = 6.3 Hz), δ 2.02 (m), δ 2.06 (m) and δ 3.95 (d, J = 7.0 Hz) indicated the xanthone ring to be substituted by a prenyl and a geranyl group. One broad hydroxyl singlet and another broad doublet at δ6.59 and δ7.04 respectively were also observed. The methine proton of the prenyl group gave a triplet at δ5.35 (J = 7.0 Hz) while the two methines of the geranyl group resonate at δ 5.21 (t, J = 6.3 Hz) and δ 5.04 (t, J = 6.7 Hz). The DEPT experiment confirmed the presence of five methine hydrogens, five methyls, and three methylene hydrogens in the molecule indicating the xanthone rings to have only two uncoupled hydrogens. The rest of the carbons in the rings carry the one methoxy, three hydroxyls, one prenyl and one geranyl group. The ^{13}C NMR spectrum gave a total of twenty-nine carbons one of which was very downfield at δ 182.6. This was assigned to the carbonyl group of the middle ring. The methoxy carbon was observed at δ 56.0. Other carbon signals were assigned using the DEPT and HETCOR spectra. The attachment of the prenyl group at C-8 was confirmed by the HMBC spectrum which indicated a ^{3}J coupling of H-21 to C-7 and a ^{3}J coupling to C-8a. Inversely a ^{3}J coupling was also

Table 1. Proton and Carbon connectivities and their ^{2}J, ^{3}J and ^{4}J interactions obtained from ^{1}H-^{13}C HETCOR and HMBC experiments for cuneifolin

Proton resonance	Connectivity	^{2}J	^{3}J	^{4}J
6.34 (H-2)	94.3 (C-2)	162.3 (C-3) 163.2 (C-1)	103.4 (C-9a),	
3.89 (3-OMe)	162.3 (C-3)			148.1 (C-4)
6.77 (H-7)	113.2 (C-7)	146.2(C-6), 128.7(C-8)	32.9 (C-21) 111.6 (C-8a)	
3.48 (H-11)	21.6 (C-11)		153.0 (C-10a)	
5.21 (H-12)	123.0 (C-12)			
1.75 (H-15)	17.9 (C-15)			
5.04 (H-17)	124.0 (C-17)			
3.95 (H-21)	32.9 (C-21)		113.2(C-7), 111.6(C-8a)	
5.35 (H-22)	122.4 (C-22)			
1.72 (H-24)	25.6 (C-24)			
1.83 (H-25)	16.2 (C-25)			

Table 2. LC_{50} and LC_{90} values for the crude extracts of *Garcinia cuneifolia*

Extract	LC_{50} (μg ml^{-1})[a] (95% C.L.)[b]	LC_{50} (μg ml^{-1})[a] (95% C.L.)[b]	Slope ± S.E[c]
Hexane	119.9 (112.6-126.2)	156.7 (147.1-172.5)	11.01±1.54
Ethanol	105.2 (96.8-113.2)	158.9 (144.4-183.3)	5.24±0.12

[a]LC = lethal concentration, [b]95% C.L.= confidence interval at 95% confidence level, [c]S.E = standard error.

observed between H-7 and C-21 further confirming the attachment of the prenyl group to C-8. Other ^2J couplings were also observed between H-7 and C-6 and C-8 and a ^3J to C-8a. This confirms the attachment of a proton to C-7 in contrast to an OMe group in cowanin (Pattalung P. *et al.*, 1994). The HMBC spectrum also gave a ^3J coupling between H-11 and C-10a. This means the geranyl is bonded to C-5. Couplings between the H-2 singlet and C-9a, C-3 and C-1 were also observed in the HMBC spectrum hence confirming a hydrogen to be bonded to C-2. The OMe group protons were coupled to C-4a and C-4 hence confirming it to be bonded to C-3. The HMBC spectrum also indicated a coupling between the 6-OH proton to C-12 hence indicating a hydroxyl group to be at the C-6 position. Hence a third hydroxyl group was assigned to the C-4 position.

Bioassay testings on the crude hexane and ethanol extracts of *Garcinia cuneifolia* indicated the plant to be moderately toxic to the larvae of *Aedes aegypti*. The hexane extract gave an LC_{50} value of 119.9 μg ml^{-1} while the ethanol an LC_{50} value of 105.2 μg ml^{-1}. Table 4 gives the bioassay results of these extracts.

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