Polyanxanthone A, B and C, three xanthones from the wood trunk of *Garcinia polyantha* Oliv.

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Abstract

Three xanthones, polyanxanthone A (1), B (2) and C (3) have been isolated from the methanol extract of the wood trunk of *Garcinia polyantha*, along with five known xanthones: 1,3,5-trihydroxyxanthone (4); 1,5-dihydroxyxanthone (5); 1,3,6,7-tetrahydroxyxanthone (6); 1,6-dihydroxy-5-methoxyxanthone (7) and 1,3,5,6-tetrahydroxyxanthone (8). Their structures were determined by means of 1D- and 2D-NMR techniques. Some of the above compounds were screened for their anticholinesterase activity on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

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1. Introduction

The genus *Garcinia* of the Guttiferae family is well known to be a rich source of bioactive isoprenylated xanthones (Kanda et al., 2006; Deachathai et al., 2006; Komguem et al., 2005) and benzophenones (Nilar et al., 2005; Williams et al., 2003). In continuation of our search for bioactive substances from *Garcinia* species, we have investigated the methanol extract of the wood trunk of *Garcinia Polyantha* Oliv, a tree distributed in the lowland tropical rainforest of west, east and central Africa (Ampofo and Waterman, 1986; Brehaut, 1975). This investigation led to the isolation of three new xanthones (1–3) and five known xanthones (4–8). We report herein, the structure elucidation of the above compounds along with their anticholinesterase activities.

2. Results and discussion

The methanol extract of powder wood trunk of *G. polyantha* was separated by silica gel column chromatography to give three new xanthone derivatives named polyanxanthone A (1), B (2) and C (3) along with five known xanthones: 1,3,5-trihydroxyxanthone (4) (Zhang et al., 2002); 1,5-dihydroxyxanthone (5) (Nkengfack et al., 2002); 1,3,6,7-tetrahydroxyxanthone (6) (Carpenter et al., 1969); 1,6-dihydroxy-5-methoxyxanthone (7) (Zhang et al., 2002) and 1,3,5,6-tetrahydroxyxanthone (8) (Frahm and Chandhuri, 1979).

The following spectral data showed that all the new xanthones have a prenyloxy groups at C-1 and C-5. UV
absorption of these compounds showed two strong bands at $\lambda_{\text{max}}$ 240–248 and 254–266 nm, a medium bands at $\lambda_{\text{max}}$ 302–312 nm, and weak broad bands at $\lambda_{\text{max}}$ 352–390 nm indicated the conjugated chromophore systems (Ito et al., 2003). The IR spectra showed evenly bands at $\nu_{\text{max}}$ 1656–1665 and 1560–1601 cm$^{-1}$ (Meli et al., 2005) suggesting a carbonyl and an aromatic double bonds, respectively.

Polyanxanthone A (1) was isolated as a white crystalline, mp: 135–137 °C. The HREIMS spectroscopy of this compound gave the molecular ion peak at $m/z$ 448.2277 (Calc. 448.2212) corresponding to C$_{28}$H$_{32}$O$_{5}$ formula. The $^1$H NMR spectrum showed signals due to three prenyloxy groups, respectively at $[\delta_{\text{H}}$ 4.63(2H, d, $J = 6.3$ Hz, H-1'), 5.57 (1H, t, $J = 6.3$ Hz, H-2'), 1.76 (3H, s, H-5'), 1.80 (3H, s, H-4')]; $[\delta_{\text{H}}$ 4.57 (2H, d, $J = 6.8$ Hz, H-1'), 5.52 (1H, t, $J = 6.8$ Hz, H-2''), 1.72 (3H, s, H-5''), 1.77 (3H, s, H-4'')] and $[\delta_{\text{H}}$ 4.70 (2H, d, $J = 6.6$ Hz, H-1''), 5.62 (1H, t, $J = 6.6$ Hz, H-2''), 1.75 (3H, s, H-5''), 1.80 (3H, s, H-4'')] as three substituents of xanthone nucleus. Further more, an ABX type signals, a lower field signal at $\delta_{\text{H}}$ 7.84 (1H, dd, $J = 7.8$ and 1.4 Hz) was assignable to H-8, which was affected by deshielding due to 9-C=O, and two others signals at $\delta_{\text{H}}$ 7.19 (1H, t, $J = 7.8$) and $\delta_{\text{H}}$ 7.14 (1H, dd, $J = 7.8$ and 1.4 Hz) attributed to H-7 and H-6, respectively. The remaining signals observed as meta-coupled aromatic proton at $\delta_{\text{H}}$ 6.60 (1H, d, $J = 2.2$ Hz) and $\delta_{\text{H}}$ 6.34 (1H, d, $J = 2.2$ Hz) were assignable to H-2 and H-4, respectively. HMBC correlations (Table 1) from 9-C=O, C-6, C-10a to H-8, from C-8, C-5, C-10a to H-6, from C-5, C-8a to H-7, from C-9a, C-4 to H-2 and from C-9a, C-2 to H-4 firmly confirm the location of different aromatic protons. As far as, C=H long range correlations in the HMBC spectrum (Table 1) from oxygenated quaternary carbon C-1 to H-1', from C-3 to H-1'' and from C-5 to H-1'' indicated the assignment of the location of the three prenyl groups. On the basis of these data, the structure of polyanxanthone A was determined to be (1), identified as 1,3,5-triprenyloxyxanthone.

Polyanxanthone B (2) was obtained as a white crystal, mp: 139.9–140 °C. The molecular formula was determined as C$_{23}$H$_{32}$O$_{5}$ from the HREIMS, $m/z$ 346.168 (Calc. 346.1675). It is $^1$H NMR spectrum was shown to be quite similar to that of (1), with characteristic resonances which again could be associated with two prenyloxy moieties, respectively at $[\delta_{\text{H}}$ 4.68 (2H, d, $J = 5.7$ Hz, H-1'), 5.57 (1H, t, $J = 5.7$ Hz, H-2'), 1.80 (3H, s, H-4'), 1.78 (3H, s, H-5')] and $[\delta_{\text{H}}$ 4.70 (2H, d, $J = 4.2$ Hz, H-1''), 5.62 (1H, t, $J = 4.2$ Hz, H-2''), 1.78 (3H, s, H-4''), 1.74 (3H, s, H-5'')] and three aromatic protons $[\delta_{\text{H}}$ 7.17 (1H, dd, $J = 7.8$ and 1.6 Hz, H-6), 7.21 (1H, t, $J = 7.8$ Hz, H-7) and 7.85 (1H, dd, $J = 7.8$ and 1.6 Hz, H-8)]; these were observed except for the appearance of new aromatic proton ABX system on ring C at $\delta_{\text{H}}$ 6.77 (1H, dd, $J = 8.4$ and 1.2 Hz, H-2), 7.14 (1H, dd, $J = 8.4$ and 1.2 Hz, H-4) and 7.56 (1H, t, $J = 8.4$ Hz, H-3), and the lack of a typical prenyloxy moiety aforementioned in compound (1). Examination of the contour plot of an HMBC experiment (Table 1) then revealed that the ring A of (2) was identical to that of polyanxanthone A and characterized with one prenyloxy group at C-5. Therefore, polyanxanthone B (2) was identified as 1,5-diprenyloxyxanthone.

Polyanxanthone C (3) was isolated as yellow oil. This compound was showed to have the molecular formula C$_{28}$H$_{32}$O$_{5}$ by HREIMS which showed the [M+] ion peak at $m/z$ 432.1683 (Calc. 432.1679). The $^1$H NMR features were similar to those of (2), except for the appearance of prenyl group at $\delta_{\text{H}}$ 3.45 (2H, d, $J = 7.2$ Hz), 5.30 (1H, t, $J = 7.2$ Hz), 1.71 (3H, s) and 1.74 (3 H, s) instead of an aromatic proton appearing at $\delta_{\text{H}}$ 6.77 in ring C of (2). The lack of ABX system aforesaid on (2) and the presence of two aromatic protons ortho-coupled firmly indicated that H-2 was substituted by a new prenyl moiety. This hypothesis was confirmed after recording of HMBC and NOESY experiments showing on one side the expected long range correlation between H-1'' of prenyl group and C-1, C-2 and C-3 (Table 1) together with the strong nuclear overhauser effect between H-1''/H-1', and in the other side the strong nuclear overhauser between H-3/H-4, H-3/H-2' and H-1''/H-6 (Fig. 1). These results indicated the structure of polyanxanthone C (3), identified as 1,5-prenyloxy-2-(3-methylbut-2-enyl) xanthone.

2.1. Anticholinesterase activity

Compounds 1, 2, 4, 5 and 7 were screened for their cholinesterase inhibitory potential. The compounds 4, 5 and 7 showed significant inhibition against BChE with an IC$_{50}$ value of 93.0, 2.54 and 74.4 μM, respectively, while compound 2 showed significant inhibition against both AChE (IC$_{50}$ = 46.3 μM) and BChE (IC$_{50}$ = 25.5 μM) compared to the standard, galantamine (IC$_{50}$ = 0.5 μM and 8.5 μM, respectively). Compound 1 showed 41.8% and 7.0% inhibition against AChE and BChE, respectively, at the concentration of 0.2 mM.

3. Experimental

3.1. General procedure

The melting points were determined on a micro melting point apparatus (Yanaco MP-S3 apparatus) and are uncorrected. Infrared spectra were obtained on a JASCO A-302 spectrophotometer using KBr pellets. Ultraviolet spectra were recorded on a Shimadzu UV 240 spectrophotometer in methanol. Mass spectra (EI and HREIMS) were measured in an electron impact mode on Varian MAT 312 spectrometers. The $^1$H NMR spectra were registered on a Bruker Avance AMX 500 NMR spectrometer with tetramethylsilane (TMS) as an internal standard; while $^{13}$C NMR spectra were recorded on a Bruker Avance AMX 500 NMR spectrometer operating at 125 MHz using CDCl$_3$ as solvent. Methyl, methylene and methine carbons were distinguished by DEPT experiments. Homonuclear
Table 1

| Position | 1H (m, J (Hz)) | 13C | HMBC | 2 | 1H (m, J (Hz)) | 13C | HMBC | 3 | 1H (m, J (Hz)) | 13C | HMBC |
|---------|----------------|-----|------|---|----------------|-----|------|---|----------------|-----|------|
| 1       | 6.60 (d, 2.2)  | 161.1 | C-3, C-9a, C-4, C-1 | | | | | | | | |
| 2       | 6.77 (dd, 8.4 and 1.2) | 159.6 | C-1, C-9a, C-4 | | | | | | | | |
| 3       | 7.14 (dd, 8.4 and 1.2) | 157.9 | C-1, C-9a, C-4a | | | | | | | | |
| 4       | 7.14 (dd, 8.4 and 1.2) | 145.7 | C-1, C-9a, C-4a | | | | | | | | |
| 5       | 7.17 (dd, 8.4 and 1.2) | 147.3 | C-2, C-9a, C-4a | | | | | | | | |
| 6       | 7.71 (dd, 7.8 and 1.6) | 116.6 | C-8, C-5, C-10a | | | | | | | | |
| 7       | 7.21 (t, 7.9) | 123.1 | C-5, C-8a | | | | | | | | |
| 8       | 7.85 (dd, 7.8 and 1.6) | 117.8 | C-9, C-10a, C-6 | | | | | | | | |
| 9       | 7.67 (d, 1.2) | 144.5 | C-2 | | | | | | | | |
| 10      | 7.14 (dd, 7.8 and 1.4) | 116.6 | C-10a, C-8, C-5 | | | | | | | | |
| 11      | 7.21 (t, 7.9) | 123.1 | C-5, C-8a | | | | | | | | |
| 12      | 7.85 (dd, 7.8 and 1.6) | 117.8 | C-9, C-10a, C-6 | | | | | | | | |

1H and 13C NMR data of polyanxanthone A (1), B (2) and C (3) (500, 125 MHz) in CDCl3

1H connectivities were determined by using the COSY experiment. One-bond 1H-13C connectivities were determined with HMOC gradient pulse factor selection. Two and three bond 1H-13C connectivities were determined by HMBC experiment. Chemical shifts were reported in δ (ppm) and coupling constants (J) were measured in Hz. TLC was performed on precoated silica gel cards (E. Merck), spots were viewed under ultraviolet light at 254 nm for fluorescence quenching spots and at 366 nm for fluorescent spots and stained by spraying with a solution of ceric sulphate (0.2%) in H2SO4 (5%). For column chromatography, silica gel (E. Merck, 230–400 mesh) were used. All reagents used were of analytical grades.

3.2. Plant material

The wood trunk of G. polyantha was collected from Mt Kala, central-province Cameroon in August 2003, and identified by Dr. Tchiengue Batelheamy of the Cameroon Nation Herbarium (Yaoundé), where a voucher specimen (21337/SRF/Cam/Mt Kala) was deposited.

3.3. Extraction and isolation

Air dried and ground wood trunk of G. polyantha (3.5 kg) was macerated in methanol for three days at room temperature and the fractions were concentrated under reduce pressure to yield 83 g of methanol extract. The methanol extract was subjected to column chromatography eluting with hexane, hexane-CH2Cl2, CH2Cl2-MeOH in increasing order of polarity to afford fractions A-D. The mixture of fraction A and B (4.2 g) obtained with Hexane-CH2Cl2 (9.5:0.5 and 8.5:1.5, respectively) was purified by column chromatography over silica gel (5 × 80 cm) eluted with a mixture of hexane-CH2Cl2 in increasing order of polarity to yield compounds 1 (16 mg), 4 (122 mg) and...
three sub fractions (B₁–B₃). The sub fraction B₂ (1.4 g) obtained with hexane-CH₂Cl₂ (9.0:1.0) was further purified by silica gel column chromatography (3 × 50 cm) with hexane-CH₂Cl₂ (9.3:0.7) to give compound 2 (15 mg) and 5 (47 mg), and the sub fraction B₁ was purified by preparative TLC (hexane-CH₂Cl₂ (9.5:0.5) to give compound 3 (14 mg). Fraction C (1.6 g) obtained with hexane-CH₂Cl₂ (8.0:2.0) was purified by column chromatography over silica gel (3 × 50 cm) eluted with a mixture of hexane-CH₂Cl₂ in increasing order of polarity to yield compounds 6 (14 mg), 8 (14 mg) and 7 (37 mg). Finally, fraction D was found to contain mainly very polar compounds.

### 3.3.1. Polyanxanthone A (1)

Whitish crystal, m.p.: 135–137 °C. UV (MeOH) λ max (logε): 303 (2.5), 247 (3.04), 275 (2.35), 225 (2.75). IR (KBr) ν max cm⁻¹ 3626, 2914, 1656, 1601, 1289, 1159, 1101, 956, 670. EIMS: m/z 448 [M⁺] (6.86), 379 (4), 312 (16), 244 (69), 69 (100). HR-EIMS: m/z 448.2277 (calc. 448.2250 for C₂₈H₃₂O₅). ¹H (500 MHz) and ¹³C (125 MHz) NMR, see Table 1.

### 3.3.2. Polyanxanthone B (2)

White crystal; m.p.: 139.9–140 °C. UV (MeOH) λ max (logε): 353 (2.53), 246 (3.24), 202 (3.09). IR (KBr) ν max cm⁻¹ 3629, 2972, 1601, 1490, 1379, 821, 767. EIMS: m/z 364 [M⁺] (2.1), 296 (100), 119 (37). HR-EIMS: m/z 364.1686 (calc. 364.1675 for C₂₃H₂₄O₄). ¹H (500 MHz) and ¹³C (125 MHz) NMR, see Table 1.

### 3.3.3. Polyanxanthone C (3)

Yellow oil. UV (MeOH) λ max (logε): 389 (2.05) 340 (1.70), 297 (2.08), 255 (2.86), 203 (3.05). IR (KBr) ν max cm⁻¹ 3566, 2972, 1656, 1566, 1432, 1268, 1227, 1101, 956, 677. EIMS: m/z 432 [M⁺] (2.07) 364 (10), 296 (34), 253 (25), 241 (77), 69 (100). HR-EIMS: m/z 432.1234 (calc. 432.1237 for C₂₉H₃₂O₄). ¹H (500 MHz) and ¹³C (125 MHz) NMR, see Table 1.

### 3.4. Anticholinesterase assays

Electric-eel AChE (EC 3.1.1.7), horse-serum BChE (EC 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5'-dithio-bis-nitrobenzoic acid (DTNB) and galanthamine were purchased from the Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade. AChE and BChE inhibiting activities were measured by the spectrophotometric method developed by Ellman (Ellman et al., 1961). Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates to assay AChE and BChE, respectively. The reaction mixture contained 130 µl (100 mM) sodium phosphate buffer (pH 8.0), 20 µl of DTNB, 10 µl of test compound solution and 20 µl of AChE or BChE solution, which were mixed and incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 20 µl acetylthiocholine or butyrylthiocholine, respectively. The hydrolysis of acetylthiocholine and butyrylthiocholine were monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine and butyrylthiocholine, respectively at a wavelength of 412 nm (15 min). Test compounds and the positive control (galanthamine) were dissolved in EtOH. All the reactions were performed in triplicate in 96-well micro-plates in SpectraMax 340 (molecular Devices, USA). The concentrations of test

![Fig. 1. NOESY correlations of 3.](image-url)
compounds that inhibited the hydrolysis of substrates (acetylthiocholine and butyrylthiocholine) by 50% (IC$_{50}$) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC$_{50}$ values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA.).

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