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TWO PRENYLATED ANTHRONES IN *HARUNGANA MADAGASCARIENSIS*

MUNEKAZU IINUMA, HIDEKI TOSA, TETSURO ITO, TOSHIYUKI TANAKA and MOHAMMAD AQIL*

Department of Pharmacognosy, Gifu Pharmaceutical University, 6 - 1 Mitahora-higashi 5 chome, Gifu 502, Japan; *Department of Chemistry, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

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Key Word Index—*Harungana madagascariensis*; Guttiferae; anthrone; harunganol A; harunganol B; 2-(3-methyl-1-butenyl)-1,8-dihydroxy-3-methoxy-6-methylanthrone; 1,3,8-trihydroxy-4,5-diisoprenyl-6-methylanthrone; 1,3,8-trihydroxy-4,5,7-triisoprenyl-6-methylanthrone; 1,5,6-trihydroxy-7-methoxyxanthone.

Abstract—Two new anthrones were isolated from *Harungana madagascariensis* in addition to a known anthrone, two xanthones, four anthraquinones and two flavonoids. The structures of the two anthrones were determined to be 1,3,8-trihydroxy-4,5-diisoprenyl-7-methylanthrone (harunganol A) and 1,3,8-trihydroxy-4,5,7-triisoprenyl-7-methylanthrone (harunganol B) by spectroscopic analysis. The other anthrone, 1,8-dihydroxy-2-isoprenyl-3-methoxy-6-methylanthrone which has been once derived from natural product, and one xanthone, 1,5,6-trihydroxy-7-methoxyxanthone which has been synthesized earlier were isolated for the first time as genuine natural products.

INTRODUCTION

In our continued phytochemical studies on plants of the Guttiferae with bioactive constituents (*Garcinia subellipitica* [1], *G. mangostana* [2] and *Calophyllum inophyllum* [3]), *Harungana madagascariensis* Lam. ex Poir. (Guttiferae), native to Africa and Madagascar, was examined. The leaves, roots and stems have been used for various medicinal purposes in Africa [4]. Although some chemical aspects of *H. madagascariensis* have been reported [5, 6], the detailed chemistry is still unknown. We report here the isolation and characterization of two new anthrones in *H. madagascariensis* along with nine known compounds.

RESULTS AND DISCUSSION

The roots and branches of *H. madagascariensis* were extracted separately after being dried and ground with benzene, acetone and 70% MeOH in succession. The leaves were also extracted with acetone, and the extract suspended into water as partitioned with benzene and EtOAc, successively. Each extract was repeatedly chromatographed on silica gel and Sephadex LH - 20 to give compounds **1–5** (from the benzene extract of roots and branches), **6** and **7** (the acetone extract of roots and branches), **8** (the benzene extract of branches), **9** (the benzene soluble extract of leaves), and **10** and **11** (the EtOAc extract of leaves).

Compound **1**, obtained as a pale yellow amorphous powder, reacted positively to $FeCl_3$ and Gibbs tests. The $[M^+]$ at *m/z* 338.1504 in the high resolution EIMS corresponds to $C_{21}H_{22}O_4$. The ^{13}C NMR and IR spectrum showed the presence of a carbonyl group (δ_C192.3, 1615 cm^{-1}). In the ^1H NMR spectrum, a two-proton singlet was observed at δ_H 4.21 due to a methylene group. These findings and the UV spectral data suggested that **1** is an anthrone derivative. In the ^1H NMR spectrum, the presence of two chelated hydroxyl groups (δ_H12.29 and 13.16), a methoxyl (3.92) and an aromatic methyl group [2.34 (*br s*)] in addition to a 3-methyl-1-butenyl group [δ1.12 (6H, *d*, *J* = 6.8 Hz, Me × 2), 2.48 (1H, *m*, CH), 6.57 (1H, *d*, *J* = 16.1 Hz, *trans*-olefinic proton) and 6.69 (1H, *dd*, *J* = 16.1, 6.8 Hz, *trans*-olefinic proton)] was exhibited. The spectrum further showed the presence of an aromatic proton [δ6.40 (1H, *br s*)] and *meta*-coupled protons (δ6.65 and 6.67), both of which were observed in a broad singlet because of a long-range coupling with the aromatic methyl group (δ_H2.34). These results indicated that the partial structure of **1** was expanded to a 1,8-dihydroxy-6-methylanthrone. The positions of methoxyl and the 3-methyl-1-butenyl group were decided as follows. NOEs were observed between the methylene protons (δ 4.21) and one of the *meta*-coupled protons (δ_H6.65) and an aromatic proton (6.40) (Fig. 1). An NOE was also observed between the aromatic proton (δ6.40) and the methoxyl group (δ3.92). The structure of **1** was then characterized as 2-(3-methyl-1-butenyl)-1,8-dihydroxy-3-methoxy-6-methylanthrone, which was confirmed by the HMBC experiment (Fig. 1). Although this compound was previously derived from vismione A [7], this is the first isolation from a natural source. There is a possibility that **1** is an artefact of vismione A by use of silica gel [7], but we definitely confirmed the presence of **1** in the benzene extract roots on TLC.

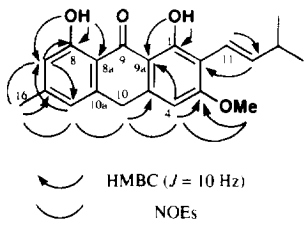

Fig. 1. HMBC and NOE experiments of **1**.

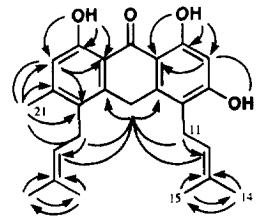

Fig. 2. HMBC and NOE experiments of **2**.

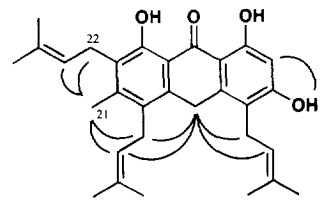

Fig. 3. NOE experiments of **3**.

Compound **2**, harunganol A, was obtained as a pale yellow amorphous powder which gave a positive $FeCl_3$ reaction. The high resolution EIMS showed the molecular ion peak at *m/z* 392.1975 which corresponds to $C_{25}H_{28}O_4$. Its UV, IR and ^1H NMR spectrum were suggestive that **2** was also an anthrone derivative. The UV absorptions were closely similar to those of γ-hydroxyanthrone B, a 1,3,8-trioxygenated anthrone derivative isolated from *Vismia* species [8]. The ^1H NMR spectrum showed the presence of three hydroxyl groups [δ9.58 (1H, *br s*), 12.46 and 12.65 (1H each, *s*, chelated)], an aromatic methyl group (δ2.34) and two aromatic protons [δ6.43 and 6.72 (1H each, *s*)], in addition to methylene protons due to an anthrone skeleton [δ4.18 (2H, *s*)] and two isoprenyl groups. These results suggested that **2** is a 1,3,8-trihydroxyanthrone with two isoprenyl groups. The whole structure was elucidated by the following NOE experiments (Fig. 2). NOEs were observed between the methylene proton of the skeleton (δ4.18) and two methine protons on the isoprenyl groups (δ4.96 and 5.06), between the aromatic methyl group (δ2.34) and one of the aromatic protons at δ6.72 and the methylene protons on the isoprenyl group (δ3.43), and between the irradiated hydroxyl group (δ9.58) and the anthrone aromatic proton (δ6.43). Thus harunganol A is 1,3,8-trihydroxy-4,5-diisoprenyl-6-methylanthrone (**2**), and this structure was substantiated by the HMBC spectrum (Fig. 2).

Compound **3**, harunganol B, obtained as a pale yellow amorphous powder, reacting positively with $FeCl_3$. The $[M^+]$ at *m/z* 460.2585 in the high resolution EIMS corresponds to $C_{30}H_{36}O_4$. Its IR and UV spectrum suggested that **3** is an anthrone. In particular, its UV absorption conspicuously resembled that of **2**, which suggested that **3** had the same oxidation pattern. The ^1H NMR spectrum was also similar to **2** except for disappearance of an aromatic proton and newly appeared signals due to another isoprenyl group attached to C-7. The structure of harunganol B was finally characterized as 1,3,8-trihydroxy-4,5,7-triisoprenyl-6-methylanthrone (**3**) after the NOE experiments presented in Fig. 3.

Compound **7** was obtained as a yellow amorphous powder which positively reacted with $FeCl_3$ and Gibbs. In the high resolution EIMS, the $[M^+]$ at *m/z* 274.0490 was observed which showed the molecular formula is $C_{14}H_{10}O_6$. The UV and IR spectrum indicate that **7** is a xanthone. In the ^1H NMR spectrum, the presence of three hydroxyls [δ9.98 (2H, *br s*) and 12.99 (1H, *s*, chelated), a methoxyl group (δ3.91) and an aromatic proton [δ7.12 (1H, *s*)] was confirmed in addition to the presence of three protons in an ABC system [δ6.77 (1H, *br d*, J = 8.3 Hz), 7.05 (1H, *br d*, J = 7.8 Hz) and 7.67 (1H, *dd*, J = 8.3, 7.8 Hz)]. All protonated carbons were assigned by CH-COSY spectrum (Table 1). In the HMBC spectrum (Fig. 4), the chelated hydroxyl group (δ_H12.99) was correlated to three aromatic carbons (δ_C107.6, 109.5 and 160.8), one of which (δ109.5) was further correlated to the proton (δ6.77) in the CH-COSY spectrum. The aromatic proton at δ7.12 caused cross peaks to the carbonyl group (δ180.6) and an aromatic carbon with *O*-function (δ146.2), which indicated that the aromatic proton was located at a *peri*-position to the carbonyl group. The latter carbon (δ146.2) was also correlated to the methoxyl group (δ3.91). The structure of **7** is 1,5,6-trihydroxy-7-methoxyxanthone. Although **7** has already been synthesized [9], this is the first report as a natural product.

Compounds **4–6** and **8–11** were determined by the spectral analysis to be madacascin (emodin 3-isoprenyl ether (**4**) [5], chrysophanol (**5**), 1,7-dihydroxyxanthone (**6**), vismiaquinone (1.8-dihydroxy-7-(3-methyl-1-butenyl)-6-methoxy-3-methylanthraquinone) (**8**) [10], aloe-emodin ω-acetate (**9**), astilbin (taxifolin 3-*O*-rhamnoside) (**10**) and quercetin (**11**), respectively.

EXPERIMENTAL

Plant material. The roots, branches and leaves of *H. madagascariensis* were collected in Nigeria in September, 1994. The voucher specimens are deposited in the Herbarium of Gifu Pharmaceutical University.

Extraction and isolation. The dried and ground roots (500 g) were successively extracted with benzene (2 l × 4) (the weight of extract after evaporation: 4.5 g), acetone (2 l × 4) (5 g) and 70% MeOH (2 l × 4) (40 g) for 24 hr at

Table 1. ^{13}C NMR spectral data of compounds **1–3** and **7**

Carbon No. (solvent)	**1** ($CDCl_3$)	**2** (acetone – d_6)	**3** (acetone – d_6)	**7** (DMSO – d_6)
1	162.2	162.7	164.8	160.8
2	113.7	100.0	102.1	109.5
3	162.8[a]	161.5	163.5	136.2
4	101.3	117.0	119.0	106.9
5	119.5	127.6	129.6	133.2
6	147.0	145.2	145.7	142.8[j]
7	115.9[k]	115.7	127.4	146.2
8	162.9[a]	160.0	160.0	95.3
9	192.3	191.7	193.9	180.6
10	33.2	28.1	28.1	
4a	141.1[b]	140.8	143.0	155.6
8a	110.3	112.3	113.8	111.3
9a	112.5	107.9	110.1	107.6
10a	141.5[b]	138.0	137.4	142.5[j]
11	115.9[k]	22.6	24.5	
12	143.0	120.4[c]	122.3[g]	
13	33.1	131.0[d]	132.0[h]	
14	22.7	23.9[e]	25.5[l]	
15	22.7	16.1[f]	17.7[i]	
16	22.0	25.7	25.1	
17		120.7[c]	122.4[g]	
18		131.8[d]	133.1[h]	
19		23.8[e]	25.5[l]	
20		16.1[f]	17.8[i]	
21		19.0	16.5	
22			29.7	
23			123.1[g]	
24			133.7[h]	
25			25.5[l]	
26			17.9[i]	
OMe	55.8			55.9

[a–j]: interchangeable between same letters, [k] and [l]: overlapping.

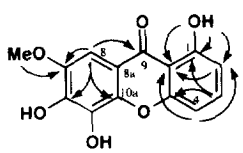

Fig. 4. HMBC spectrum (J = 10 Hz) of **7**.

room temperature. The branches (950 g) were extracted in the same way; benzene (2.5 l × 4) (5 g), acetone (2.5 l × 4) (12 g) and 70% MeOH (2.5 l × 4) (70 g) extract. An aliquot of the benzene extract of the roots (3.5 g) was subjected to vacuum liquid chromatography (VLC) on silica gel with a benzene–acetone system to give eight fractions (fr. 1–8). Compound **4** (8 mg) was obtained by recrystallization (MeOH) from fr. 1 (benzene 100%), and the residue was further purified by VLC eluted with *n*-hexane–EtOAc system to give **5** (8 mg) (25:1). Furthermore, a benzene eluent (fr. 2) was rechromatographed on silica gel with benzene to give five fractions (fr. 2-1–2-5). The fr. 2-2 was purified by Sephadex LH-20 (MeOH) to give **3** (3 mg), and the fr. 2-3 was subjected to Sephadex LH-20 (acetone) to give **1** (7 mg) and **2** (5 mg). The benzene extract of branches (7 g) was separated in a similar way to give **1** (2 mg), **2** (15 mg), **3** (20 mg), **4** (2 mg), **5** (10 mg), **8** (1 mg). On the other hand, the acetone extract of the branches (4 g) was chromatographed on silica gel by use of VLC eluted with benzene–acetone system. From a benzene–acetone (20:1) eluent, a crude amorphous powder containing **6** was obtained. This amorphous powder was purified by PTLC (benzene–acetone = 10:1) to give **6** (5 mg). From a benzene–acetone (10:1) eluent, **7** (8 mg) was obtained after recrystallization. These two compounds [**6** (15 mg) and **7** (5 mg)] were obtained from the acetone extract of the branches (9 g) by similar purification. The acetone extract of leaves (40 g) suspended into water was partitioned with benzene (1.5 l × 4) and EtOAc (1.5 l × 4) with succession. The benzene (9 g) and the EtOAc (8 g) soluble extract were repeatedly chromatographed on silica gel and Sephadex LH-20 to give **9** (8 mg) from the benzene extract, and **10** (120 mg) and **11** (5 mg) from the EtOAc extract.

Compound **1**. A pale yellow amorphous powder. High resolution (HR) EI-MS: *m/z* 338.1504 (calcd. 338.1518 for $C_{21}H_{22}O_4$); EI-MS *m/z* (rel. int.): 338 (M^+, 53), 323 (28), 295 (100), 283 (51), 280 (7), 270 (5), 264 (4), 154 (4), 149 (6); IR *v* (cm^{-1}, KBr): 2950, 2860, 1615, 1595; UV λ (nm, MeOH): 227, 272, 335sh, 354; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (6H, *d*, *J* = 6.8 Hz, H-14, 15), 2.34 (3H, *br s*, H-16), 2.48 (1H, *m*, H-13), 3.92 (3H, *s*, OMe), 4.21 (2H, *br s*, H-10), 6.40 (1H, *br s*, H-4), 6.57 (1H, *d*, *J* = 16.1 Hz, H-11), 6.65 (1H, *br s*, H-5), 6.67 (1H, *br s*, H-7), 6.69 (1H, *dd*, *J* = 16.1, 6.8 Hz, H-12), 12.29 (1H, *s*, C_8-OH), 13.16 (1H, *s*, C_1-OH).

Compound **2** (*harunganol A*). A pale yellow amorphous powder. HR EI-MS: *m/z* 392.1975(calcd. 392.1978 for $C_{25}H_{28}O_4$); EIMS *m/z* (rel. int.): 392 (M^+, 18), 336 (73), 321 (13), 319 (7), 306 (7), 293 (12), 280 (100), 268 (9); IR *v* (cm^{-1}, KBr): 3350, 2995, 2930, 1640, 1615, 1600, 1580; UV λ (nm, MeOH): 225, 258, 274, 308, 366; ^{1}H NMR (400 MHz, acetone-d_6): δ 1.69, 1.72 (3H each, *s*, H-15, 20), 1.84, 1.85 (3H each, *s*, H-14, 19), 2.34 (3H, *s*, H-21), 3.43 (4H, *m*, H-11, 16), 4.18 (2H, *s*, H-10), 4.96, 5.06 (1H each, *t* like *m*, H-12, 17), 6.43 (1H, *s*, H-2), 6.72 (1H, *s*, H-7), 9.58 (1H, *br s*, C_3-OH), 12.46 (1H, *s*, C_8-OH), 12.66 (1H, *s*, C_1-OH).

Compound **3** (*harunganol B*). A pale yellow amorphous powder. HR-EIMS: *m/z* 460.2585 (calcd. 460.2613 for $C_{30}H_{36}O_4$); EIMS *m/z* (rel. int.): 460 (58), 417 (8), 404 (75), 389 (18), 387 (12), 371 (6), 361 (100), 349 (76), 343 (7), 335 (48), 319 (10), 305 (35), 293 (23), 230 (13), 265 (7); IR *v* (cm^{-1}, KBr): 3360, 2930, 2850, 1605; UV λ (nm, MeOH): 222*sh*, 260, 278, 313, 368; ^{1}H NMR (400 MHz, acetone-d_6): δ 1.67, 1.68 and 1.72 (3H each, *s*, H-15, 20, 26), 1.81, 1.84, 1.86 (3H each, *s*, H-14, 19, 25), 2.32 (3H, *s*, H-21), 3.46 (6H, *m*, H-11, 16, 22), 4.17 (2H, *s*, H-10), 4.95, 5.06, 5.09, (1H each, *t* like *m*, H-12, 17, 23), 6.43 (1H, *s*, H-2), 9.56 (1H, *br s*, C_3-OH), 12.68 (1H, *s*, C_8-OH), 13.04 (1H, *s*, C_1-OH).

Compound **7**. A yellow amorphous powder. HR-EIMS: *m/z* 274.0490 (calcd. 274.0477 for $C_{14}H_{10}O_6$); EIMS *m/z* (rel. int.): 274 (M^+, 100), 259 (39), 231 (24), 203 (16), 137 (6), 129 (6); IR *v* (cm^{-1}, KBr): 3505, 1655, 1615, 1590; UV λ (nm, MeOH): 210*sh*, 221, 235*sh* 245*sh*, 253, 265*sh*, 272*sh*, 329, 362*sh*; ^{1}H NMR (400 MHz, DMSO-d_6): 3.91 (3H, *s*, C_7-OMe), 6.77 (1H, *br d*, *J* = 8.3 Hz, H-2), 7.05 (1H, *br d*, *J* = 7.8 Hz, H-4), 7.12 (1H, *s*, H-8), 7.67 (1H, *dd*, *J* = 8.3, 7.8 Hz, H-3), 9.98 (2H, *br s*, OH × 2), 12.99 (1H, *s*, C_1-OH).

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