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PREGNANES AND TRITERPENOID HYDROPEROXIDES FROM THE LEAVES OF *AGLAIA GRANDIS*

AKIRA INADA,* HIROKO MURATA, YUKA INATOMI, TSUTOMU NAKANISHI and DEDY DARNAEDI†

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka, 573-01, Japan; † Herbarium Bogoriense, Jalan Ir. H. Juanda, Bogor 16122, Indonesia

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Abstract—Three pregnanes and two known cycloartane-type triterpenoid hydroperoxides were isolated from the leaves of *Aglaia grandis*. Their structures were determined using ^{1}H, ^{13}C and 2D NMR techniques. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

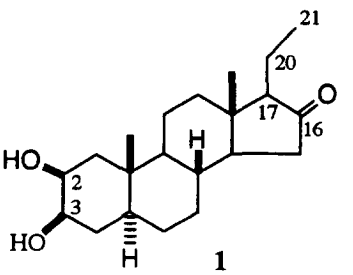
As part of our studies on the constituents of meliaceous plants [1, 2], we have now examined the leaves of *Aglaia grandis* Korth. As a result, three new pregnanes (**1**–**3**) and two known cycloartane-type triterpenoid hydroperoxides (**4**, **5**) were isolated. This paper gives the structural elucidations of these compounds.

RESULTS AND DISCUSSION

After column chromatography and HPLC separations of the hexane-soluble part of a methanol extract, three pregnanes (**1**–**3**) were isolated, together with two known cycloartane-type triterpenoid hydroperoxides (**4**, **5**). Identifications of **4** and **5** were achieved by comparison with previously reported spectroscopic data [3, 4]. Compound **1** exhibited a molecular formula of $C_{21}H_{34}O_3$ (HR-EIMS) and had IR absorptions at 3400 and 1730 cm^{-1} due to hydroxyls and a five-membered ring ketone. The ^{1}H NMR spectrum of **1** showed the presence of two tertiary methyls (δ 0.69, 1.05) and one triplet methyl (δ 1.02, J = 7.3 Hz). Based on the 2D-NMR (COSY and NOESY) results, the signals at δ 3.65 (*ddd*, J = 10.9, 4.0, 2.8 Hz) and 4.04 (*dt*, J = 4.0, 2.4), were assigned to one axial and one equatorial adjacent oxymethine protons, respectively, and each proton was further coupled with a methylene group. On the basis of the precise ^{1}H and ^{13}C NMR analyses, compound **1** was a pregnane having either a 2β,3β or 2α,3α-glycol in the A ring. Confirmation for the 2β,3β-glycol structure came from the following evidence. From the COSY spectrum, a long range (W-shape) coupling between H-2 and H-4 was observed [5]. Further, in the ^{1}H NMR spectrum, the H_3-19 group (δ 1.05) was shifted downfield by its axial 2-OH (1,3-diaxial relation) compared with that of 2α, 3α-dihydroxy pregnane [6]. Finally, the chemical shifts of C-2 and C-3 in **1** (δ 70.1 and 72.4) differ from those of 2α,3α (and also 2β,3α or 2α,3β)-dihydroxy steroids [6, 7]. These data can be reasonably accounted for by the proposed 2β,3β-glycol structure. The A/B ring junction was deduced as *trans* (i.e. 5α-pregnane series) by the chemical shift value of the C-19 methyl carbon since the C-19 methyl carbon signal of a A/B *trans*-steroid is shifted upfield by about 10–12 ppm compared with that of the corresponding A/B *cis*-steroid, the C-19 methyl resonance of which appears around δ 22–24 [7, 8]. In the ^{13}C NMR spectra of **1**, the C-19 methyl carbon signal, confirmed by CH-COSY and HMBC experiments, appeared at δ 14.5, indicating that **1** belongs to the 5α-pregnane series. In addition, the chemical shifts of other carbons on rings A and B were similar to other A/B *trans*-steroids [7–9]. The ketone group in **1** was located at C-16 from the HMBC experiments since H_2-15 (δ 1.75 and 2.20) and H-17 (δ 1.66) showed prominent cross-peaks with the carbonyl carbon (δ 219.6). Finally, detailed comparison of the ^{13}C NMR spectral data of **1** and other 16-keto steroids [5, 8, 10] confirmed the structure of compound **1** as 2β,3β-dihydroxy-5α-pregnane-16-one.

Compounds **2** and **3** showed both hydroxyl and α,β-unsaturated ketone absorptions at 3400 cm^{-1} and 1705 cm^{-1}, respectively, in their IR spectra. HR-EI mass spectroscopy showed **2** and **3** to possess the same molecular formula, $C_{21}H_{32}O_3$. In addition, both steroids gave a common and significant fragment ion at m/z 317 ([M − Me]$^+$; **2** (77%), **3** (base peak)) due

* Author to whom correspondence should be addressed.



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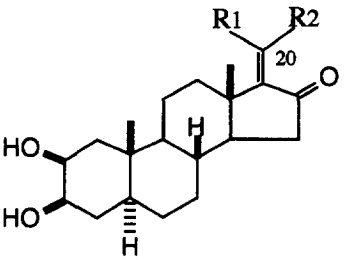

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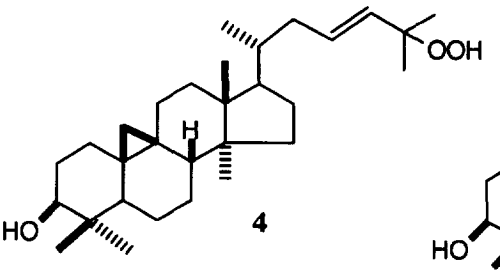
3: R1 = H, R2 = Me



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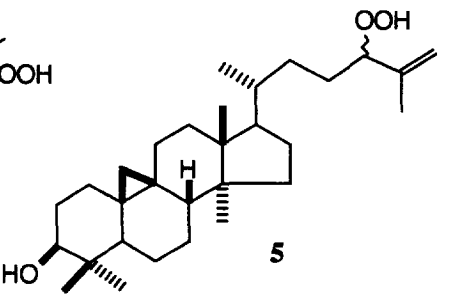

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to a typical and characteristic cleavage observed in 16-keto-Δ^{17}(20) pregnanes [8, 11, 12]. Placement of the double bond between C-17 and C-20 was further established as follows. Both compounds showed signals for three methyls, two of them tertiary (**2**, δ 1.01 and 1.06; **3** δ 0.91 and 1.05) and the other a vinylic methyl [**2**, δ 1.84 (*d*, J = 7.7 Hz) and **3**, δ 2.07 (*d*, J = 7.3 Hz)]. In addition, there was one olefinic proton as a quartet [**2**, δ 6.49 (J = 7.7 Hz) and **3**, δ 5.69 (J = 7.3 Hz)], indicating that the vinylic methyl and the olefinic proton are attached to the same C-20 carbon. The *Z*/*E* configuration of the side chain moiety and the position of the ketone function in **2** and **3** were deduced as follows. In **2**, the olefinic proton is deshielded due to the proximity of the carbonyl and appeared at δ 6.49. In **3**, this proton is oriented away from the carbonyl and appeared at higher field (δ 5.69) compared with **2**. Instead, the vinylic methyl was shifted downfield by about 0.2 ppm in **3** compared with that of **2**. The chemical shifts and coupling constants of the side chain moiety in both compounds were very similar to those of other 16-keto-Δ^{17}(20)-*Z* or *E* pregnenes [8, 12]. The ^{13}C NMR data for ring D and the side chain carbons were in good agreement with the proposed structure. The chemical shifts of the oxymethine protons (**2** δ 3.66 and 4.04; **3** δ 3.65 and 4.04) and oxymethine carbons [**2** and **3** δ 70.1 and 72.3] were essentially the same with those of **1**, suggesting the presence of the 2β,3β-glycol moiety in both compounds. Finally, from a detailed comparison of the ^{13}C NMR spectral data of both compounds with those of **1** and other Δ^{17}(20)-5α steroids [8, 10, 12], the structures of **2** and **3** were established as 2β,3β-dihydroxy-5α-pregn-17(20)-(*Z*)-en-16-one and 2β,3β-dihydroxy-5α-pregn-17(20)-(*E*)-en-16-one, respectively.

EXPERIMENTAL

General. Mps: uncorr; ^{1}H NMR: 400 MHz; ^{13}C NMR: 100 MHz in $CDCl_3$ and TMS as int. standard; IR: KBr discs; HPLC: JAIODS-120T column with a differential refractometer.

Plant materials. The leaves of *Aglaia grandis* Korth. were harvested in 1993 at the Herbarium Bogoriense and voucher specimens are deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Setsunan University.

Extraction and isolation. The crushed leaves (460 g) were extracted with MeOH and the solvent was evapd off. The MeOH extract (68.0 g) was suspended with H_2O and the aq. suspension was extracted with hexane and EtOAc, successively. The residue (22.4 g) obtained from the hexane layer, was chromatographed on silica gel with hexane–EtOAc containing increasing amounts of EtOAc and a fr. containing triterpenoid hydroperoxides (1.6 g), and a fr. containing pregnanes (0.9 g) were sepd in that order. Each fr. was further sepd by repeated HPLC to afford **1** (32 mg), **2** (7.5 mg), **3** (4.5 mg), **4** [3, 4] (80 mg), and **5** [3, 4] (12 mg).

2β,3β-*Dihydroxy-5*α-*pregnane*-16-*one* (**1**). Mp 136–

Table 1. ^{1}H NMR spectral data of compounds **1–3** [δ (ppm) in $CDCl_3$]*

H	1	2	3
H_2-1	1.16† 2.08 *dd* (14.5, 2.4)	1.16† 2.08 *dd* (14.5, 2.8)	1.16† 2.09†
H-2α	4.04 *dt* (4.0, 2.4)‡	4.04 *dt* (4.0, 2.8)‡	4.04 *dt* (4.0, 2.8)‡
H-3α	3.65 *ddd* (10.9, 4.0, 2.8)	3.66 *ddd* (11.3, 4.0, 2.8)	3.65 *ddd* (11.5, 4.0, 2.7)
H_2-4	1.40† 1.65†	1.38† 1.65†	1.35† 1.65†
H-5α	1.17 *tt* (11.7, 2.8)	1.17 *tt* (11.7, 2.8)	1.17 *tt* (11.9, 2.9)
H_2-15	1.75 *dd* (18.5, 13.3)	1.98 *dd* (16.9, 14.1)	2.00 *dd* (17.3, 13.9)
	2.20 *dd* (18.5, 7.3)	2.19 *dd* (16.9, 6.9)	2.18 *dd* (17.3, 7.1)
H-17	1.66†	—	—
H_3-18	0.69 *s*	1.01 *s*	0.91 *s*
H_3-19	1.05 *s*	1.06 *s*	1.05 *s*
H_2-20	1.25† 1.63†	6.49 *q* (7.7)	5.69 *q* (7.3)
H_3-21	1.02 *t* (7.3)	1.84 *d* (7.7)	2.07 *d* (7.3)

* Assignments were confirmed by ^{1}H-^{1}H COSY and HMQC experiments, and coupling constants (J in Hz) are given in parentheses.

† Both multiplicity and coupling constant were unclear, due to overlapping.

‡ A small long range coupling (W-shape) between 4α-H was further observed in the COSY spectrum.

Table 2. ^{13}C NMR spectral data of compounds **1–3** [δ (ppm) in $CDCl_3$]*

C	1	2	3	C	1	2	3
1	42.9 *t*	42.8 *t*	42.9 *t*	12	38.3 *t*	36.4 *t*	35.8 *t*
2	70.1 *d*	70.1 *d*	70.1 *d*	13	42.2 *s*	43.5 *s*	43.4 *s*
3	72.4 *d*	72.3 *d*	72.3 *d*	14	50.6 *d*	50.0 *d*	49.5 *d*
4	32.5 *t*	32.4 *t*	32.5 *t*	15	38.5 *t*	37.9 *t*	39.5 *t*
5	45.4 *d*	45.3 *d*	45.4 *d*	16	219.6 *s*	206.4 *s*	208.7 *s*
6	28.1 *t*	28.1 *t*	28.1 *t*	17	65.4 *d*	148.0 *s*	148.4 *s*
7	32.2 *t*	31.9 *t*	31.9 *t*	18	13.5 *q*	17.7 *q*	19.7 *q*
8	34.0 *d*	33.6 *d*	34.0 *d*	19	14.5 *q*	14.5 *q*	14.5 *q*
9	55.3 *d*	55.0 *d*	55.2 *d*	20	17.7 *t*	129.0 *d*	130.0 *d*
10	35.5 *s*	35.5 *s*	35.6 *s*	21	13.5 *q*	13.1 *q*	14.1 *q*
11	20.8 *t*	21.1 *t*	21.0 *t*				

* Multiplicities were determined by HMQC experiments.

138° (MeOH); $[\alpha]_D^{25}$ −98.9° ($CHCl_3$; *c* 0.23); IR ν_{max} cm^{-1}. 3400, 2910, 1730, 1045; EI- and high resolution EI-MS *m/z* (rel. int.): 334.2511 (M^+, $C_{21}H_{34}O_3$ requires 334.2508, 37), 316 (25), 248 (100), 231 (53), 230 (54), 59 (77); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

2β,3β-Dihydroxy-5α-pregn-17(20)-(Z)-*en*-16-*one* (**2**). Mp 208–211° (MeOH); $[\alpha]_D^{20}$ −108.9° ($CHCl_3$; *c* 0.10); IR ν_{max} cm^{-1}: 3400, 2900, 1705, 1635, 1045; EI- and high resolution EI-MS *m/z* (rel. int.): 332.2361 (M^+, $C_{21}H_{32}O_3$ requires 332.2350, 46), 317 (77), 314 (42), 299 (37), 234 (42), 135 (42), 83 (100); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

2β,3β-Dihydroxy-5α-pregn-17(20)-(E)-*en*-16-*one* (**3**). Mp 110–113° (MeOH); $[\alpha]_D^{20}$ −85.0° ($CHCl_3$; *c* 0.12); IR ν_{max} cm^{-1}: 3400, 2900, 1705, 1635, 1045; EI- and high resolution EI-MS *m/z* (rel. int.): 332.2348 (M^+, $C_{21}H_{32}O_3$ requires 332.2350, 35), 317 (100), 314 (20), 299 (34), 135 (28), 83 (64); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

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