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TRITERPENOIDS, LIGNANS AND A BENZOFURAN DERIVATIVE FROM THE BARK OF *AGLAIA ELAEAGNOIDEA*

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Key Word Index—*Aglaia elaeagnoidea*; Meliaceae; lignans; benzofuran; dammaranes; antifungal activity.

Abstract—One 1*H*-cyclopentatetrahydro[*b*]benzofuran, two lignans, two dammarane triterpenoids and one limonoid were isolated from the bark of *Aglaia elaeagnoidea*. The structures of the isolated compounds were established on the basis of spectral data. The lignan *trans*-2,3-bis(3,4,5-trimethoxybenzyl)-1,4-butanediol diacetate and 20*S*,24*S*-epoxy-25-hydroxymethyldammarane-3-one are new compounds. *trans*-3,4-Bis(3,4,5-trimethoxybenzyl)tetrahydrofuran has been synthesized, but not previously reported as a natural product.

INTRODUCTION

In the course of our routine screening for biological properties of plant extracts, the dichloromethane extract of the stem bark of *Aglaia elaeagnoidea*, collected in Java, showed antifungal activity against *Cladosporium cucumerinum* in a TLC bioassay [1].

Aglaia elaeagnoidea (syn. *A. roxburghiana* [2]) is a tree widely distributed in the tropical forests of Asia. It is generally found in the coastal regions. Previous studies on this plant showed the presence of bisamides in the aerial part [3] and cycloartenol derivatives [4, 5]. The genus *Aglaia* belongs to the Meliaceae, a family well studied for the presence of limonoids [6, 7]. The occurrence of antileukaemic bisamides [8], benzofurans [9] and triterpenoids [10, 11] has also been reported from this family.

Activity-guided fractionation afforded compound **6**, a 1*H*-cyclopentatetrahydro[*b*]benzofuran previously described in a species of the same genus [9]. This is the first report on the antifungal activity of **6**. The isolation provided five more compounds: two new lignans, one limonoid and two dammarane triterpenoids. Identification was carried out by EI and D/CI mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy with the aid of NOE and 2D NMR measurements.

RESULTS AND DISCUSSION

Fractionation of the bark dichloromethane extract of *A. elaeagnoidea* by column chromatography on silica gel followed by medium-pressure liquid chromatography and semi-preparative HPLC on RP-18 columns, and Sephadex LH-20 and silica gel chromatography, afforded compounds **1**–**6** (see Experimental).

The UV spectrum of **1** showed the typical pattern of non-conjugated phenyl groups at 232 and 280 nm. The EI mass spectrum exhibited a $[M]^+$ signal at *m/z* 432 together with the base peak at *m/z* 181 due to a trimethoxytropylium ion. The molecular weight of **1** was confirmed by the presence of $[M + H]^+$ and $[M + NH_4]^+$ signals at *m/z* 433 and 450, respectively, in the thermospray (TSP) mass spectrum. The ^{1}H NMR spectrum of **1** showed a singlet due to four protons at δ 6.24 ppm together with two singlets at δ 3.80 and 3.81, attributable to six methoxyl groups; these data were indicative of two 3,4,5-trimethoxyphenyl groups. Moreover, a spin system composed of two double doublets at δ 3.91 (2H, J = 6.4 and 8.7 Hz) and δ 3.53 (2H, J = 5.9 and 8.7 Hz), one multiplet at δ 2.64 attributable to four protons and a two-proton multiplet at δ 2.19, suggested that **1** was a highly symmetric 9,9′-monoepoxy lignan. The ^{13}C NMR spectrum confirmed that **1** was *trans*-3,4-bis(3,4,5-trimethoxybenzyl)-tetrahydrofuran, which has been synthesized previously in order to be tested as a potential platelet activating factor antagonist [12]. The chemical shift of C-7 and C-7′ at δ 39.8 in the ^{13}C NMR spectrum suggested that H-8 and H-8′ were in a *trans*-configuration [13].

The molecular weight of **2** was established as 534 amu by the TSP mass spectrum, which exhibited an $[M + NH_4]^+$ at *m/z* 552. The ^{1}H NMR spectrum of **2** showed some signals common to **1**, confirming the

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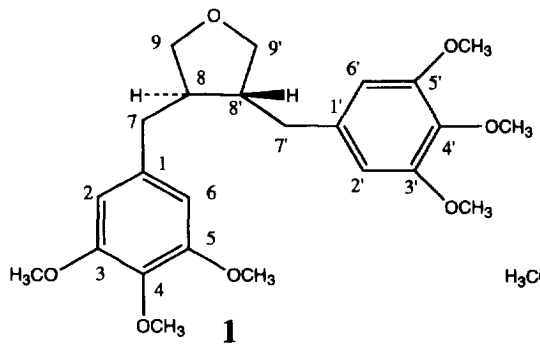

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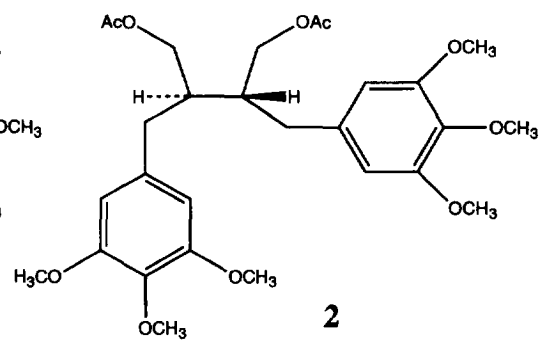

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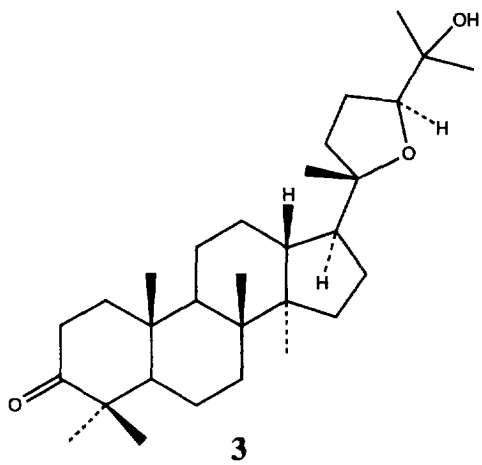

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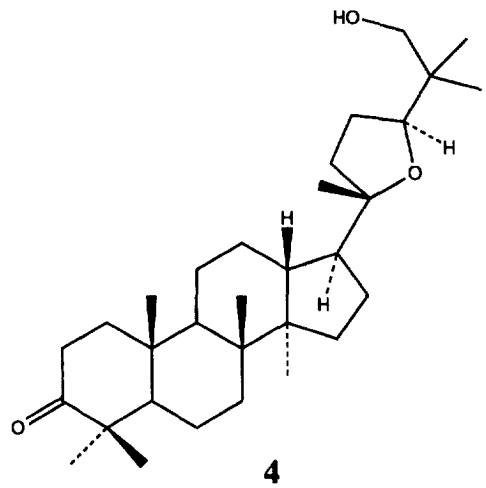

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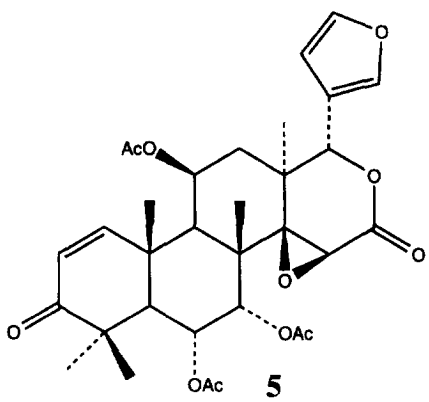

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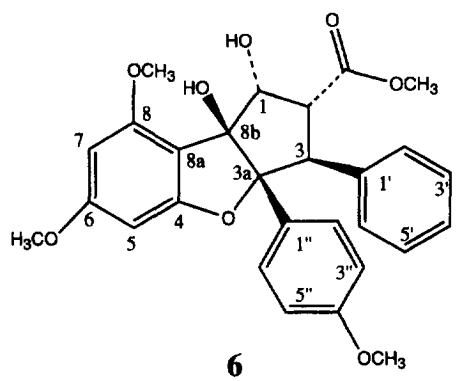

6

presence of two trimethoxyl substituted aromatic rings. Two double doublets at δ 4.25 (2H, $J = 5.6$ Hz and 11.3 Hz) and 4.00 (2H, $J = 5.3$ Hz and 11.5 Hz) and one broad doublet at δ 2.64 (4H, $J = 7.3$ Hz) indicated that the tetrahydrofuran ring was opened. Moreover, the broad singlet at δ 2.10 suggested the presence of two acetyl groups. The signals belonging to protons H-8 and H-8′ were masked by the acetyl groups. Signals of a carbonyl group at δ 170.9 and a methyl group at δ 21.0 in the ^{13}C NMR spectrum confirmed the existence of the acetyl groups. Comparison with literature spectral data of lignans [13] allowed the identification of **2** as *trans*-2,3-bis(3,4,5-trimethoxybenzyl)-1,4-butanediol diacetate, a new natural compound.

Compounds **3** and **4** presented the same characteristics on TLC analysis: absence of a UV chromophore and violet colour after addition of Godin reagent that changed to yellow. Compound **3** was identified as 20*S*, 24*S*-epoxy-25-hydroxydammaran-3-one by comparison of its spectral data with literature values [14]. This compound has been previously isolated from *Cabralea polytricha* (Meliaceae) [15].

The D/CI mass spectrum of **4** exhibited signals of $[M + NH_4]^+$ and $[M + H]^+$ ions at *m/z* 490 and 473,

respectively. The molecular weight was established as 472 amu, 14 amu more than **3**. The ^1H and ^{13}C NMR spectra of **4** were similar to those of **3**. Moreover, the ^1H NMR spectrum showed a singlet belonging to an oxygenated methylene at δ 3.49. The appearance of a CH_2 signal at δ 70.2 in the ^{13}C NMR spectrum, together with the upfield shift to δ 29.9 of the C-25, confirmed that **4** was 20*S*,24*S*-epoxy-25-hydroxymethyldammaran-3-one. Few dammarane triterpenes possessing 31 carbons have been previously described [16, 17]. However, these compounds had the extra carbon in position C-24. To our knowledge this is the first report of a dammarane with an additional methylene attached in position C-25.

The D/CI mass spectrum of **5** showed a $[M + NH_4]^+$ ion at *m/z* 616. The investigation of the ^{13}C NMR and DEPT spectra together with NOE difference experiments allowed the identification of **5** as 6α,11β-diacetoxygedunin. This limonoid was previously isolated from *Carapa guianensis* (Meliaceae) [18].

The TSP mass spectrum of **6** exhibited a $[M + H]^+$ signal at *m/z* 493. A careful study of the ^1H, ^{13}C NMR and DEPT spectra indicated that **6** was methyl rocaglate, a 1*H*-cyclopentatetrahydro[*b*]benzofuran previously isolated from *A. odorata* [9]. The structure and the assignment of the carbon signals were confirmed with the aid of HMQC and HMBC 2D NMR spectra. A series of NOE difference measurements was carried out to confirm the relative configuration on C-1, 2 and 3. These experiments established that **6** has the same relative configuration as methyl rocaglate at these positions [9].

The antifungal activities against *Cladosporium cucumerinum* and *Candida albicans* of **1**–**6** were tested in a TLC bioassay [1]. Only **6** showed activity against *C. cucumerinum* (at 2.5 μg). The amount of the reference compound, propiconazole, required to inhibit the growth of the fungi on the TLC plate was 0.01 μg.

EXPERIMENTAL

General. Mps: uncorr. TLC was carried out on silica gel precoated Al sheets (Merck). The solvent system was petrol–EtOAc (1:1). For open CC, silica gel (40–63 μm) was used. UV spectra were recorded in MeOH. Analyt. HPLC was performed on a Hewlett-Packard 1090 series II instrument equipped with a photodiode array detector. Purity of compounds was checked on Nova-Pak RP-18 columns (4 μm, 150 × 3.9 mm i.d., Waters) at a flow rate of 1 ml min^{-1}. MPLC was carried out on a LiChrosorb RP-18, 15–25 μm (Merck) stationary phase at a flow rate of 9 ml min^{-1}. Semi-prep. HPLC was performed on a LiChrosorb RP-18 column (7 μm, 250 × 16 mm i.d., Knauer) and a Nucleosil 100-7 C18 column (7 μm, 250 × 21 mm i.d., Macherey-Nagel) at a flow rate of 10 ml min^{-1}. MS were recorded on a Finnigan MAT TSQ 700 triple stage quadrupole instrument. D/CIMS: positive ion mode, NH_3 as reactant gas. TSPMS: MeCN–H_2O (4:1) 1 ml min^{-1}. An aq. soln of NH_4OAc was added (0.5 M, 0.2 ml min^{-1}) to induce ionization. A Thermospray 2 (Finnigan Mat) interface was used with the following conditions: vaporizer 100°, source 280°, aerosol 326°. ^1H and ^{13}C NMR spectra were measured at 200 and 50 MHz, respectively, in $CDCl_3$.

Plant material. Aglaia elaeagnoidea (Juss). Bth. was collected in August 1993 in Sempu Island (Java), Indonesia. The plant has been identified by the Institute Herbarium Bogoriensis, Bogor, Indonesia. A voucher specimen is deposited at the Institute Herbarium Bogoriensis.

Extraction and isolation. Dried bark of *A. elaeagnoidea* (1 kg) was successively extracted at room temp. with CH_2Cl_2 (3 × 4 l) and MeOH (3 × 4 l). The CH_2Cl_2 extract (55 g) was submitted to CC on silica gel (40–63 μm) using step gradient elution: petrol–CH_2Cl_2 (1:1, 4 l), CH_2Cl_2 (4 l), CH_2Cl_2–MeOH (49:1, 4 l) (10 ml min^{-1}); 9 frs were collected. Open CC on silica gel (40–63 μm) of fr. 8 (20 g) using $CHCl_3$–MeOH (49:1) as mobile phase (4 l, 10 ml min^{-1}) afforded 5 frs (8a–8e). MPLC on RP-18 with MeOH–H_2O (57:43, 5 l) of fr. 8d (7.27 g) afforded **1** (210 mg) and frs A (18 mg) and B (40 mg). Purification of fr. A by semi-prep. HPLC on LiChrosorb RP-18 with MeOH–H_2O (17:4) provided **5** (10 mg). Fr. B was sepd by semi-prep. HPLC on a Nucleosil C-18 column with MeOH–H_2O (3:2) giving **2** (3 mg) and **6** (30 mg). Sephadex LH-20 of fr. 8b (1.66 g) (MeOH–$CHCl_3$, 1:1) followed by CC on silica gel (40–63 μm) with petrol–EtOAc (5:2) afforded **3** (213 mg) and **4** (5 mg).

trans - 3,4 - *Bis*(3,4,5 - *trimethoxybenzyl*)*tetrahydrofuran* (**1**). Dark yellow oil. TLC: $R_f = 0.21$. UV λ_{max}^{MeOH} nm (log ε): 232 (4.15), 280 (3.75). $[\alpha]_D^{25} = +24°$ ($CHCl_3$; *c* 0.37). TSPMS *m/z* (rel. int.): 450 $[M + NH_4]^+$ (100), 433 $[M + H]^+$ (78). EIMS *m/z* (rel. int.): 432 (28), 181 (100), 167 (29), 151 (37). ^1H NMR as ref. [12]. ^{13}C NMR (50 MHz, $CDCl_3$): δ 153.1 (C-3, 3′, 5, 5′), 136.0 (C-1, 1′, 4, 4′), 105.5 (C-2, 2′, 6, 6′), 73.1 (C-9, 9′), 60.8 (MeO-4,4′), 56.0 (MeO-3, 3′, 5, 5′), 46.5 (C-8, 8′), 39.8 (C-7, 7′).

trans - 2,3 - *Bis*(3,4,5 - *trimethoxybenzyl*) - 1,4 - *butanediol diacetate* (**2**). Oil. TLC: $R_f = 0.23$. UV λ_{max}^{MeOH} nm (log ε): 230 (4.25), 278 (3.69). $[\alpha]_D^{25} = +22°$ ($CHCl_3$; *c* 0.21). TSPMS *m/z* (rel. int.): 552 $[M + NH_4]^+$ (100). ^1H NMR (200 MHz, $CDCl_3$): δ 6.25 (4H, *s*, H-2, 2′, 6, 6′), 4.25 (2H, *dd*, *J* = 5.3, 11.5 Hz, H-9a, 9′a), 4.00 (2H, *dd*, *J* = 5.6, 11.3 Hz, H-9b, 9′b), 3.80 (6H, *s*, MeO-4, 4′), 3.78 (12H, *s*, MeO-3, 3′, 5, 5′), 2.64 (4H, *d*, *J* = 7.3 Hz, CH_2-7, 7′), 2.10 (8H, *b s*, H-8, 8′, AcO-9, 9′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.9 (AcO-9, 9′), 153.1 (C-3, 3′, 5, 5′), 135.3 (C-1, 1′, 4, 4′), 105.7 (C-2, 2′, 6, 6′), 64.2 (C-9, 9′), 60.8 (MeO-4, 4′), 56.0 (MeO-3, 3′, 5, 5′), 39.5 (C-8, 8′), 35.7 (C-7, 7′), 21.0 (AcO-9, 9′).

20*S*,24*S* - *Epoxy* - 25 - *hydroxydammaran* - 3 - *one* (**3**). Powder; mp 157–161°. TLC: $R_f = 0.53$. $[\alpha]_D^{25} = +63°$

($CHCl_3$; *c* 0.2). D/CIMS *m/z* (rel. int.): 476 $[M + NH_4]^+$ (100), 459 $[M + H]^+$ (10), 441 (25). ^1H and ^{13}C NMR as ref. [14].

20S,24S-Epoxy-25-hydroxymethyldammaran-3-one (**4**). Powder; mp 173–176°. TLC: $R_f = 0.55$. $[\alpha]_D^{25} = +58$ ($CHCl_3$; *c* 0.13). D/CIMS *m/z* (rel. int.): 490 $[M + NH_4]^+$ (60), 473 $[M + H]^+$ (100), 455 (30). ^1H NMR (200 MHz, $CDCl_3$): δ 0.89, 0.95, 1.01, 1.04, 1.08 (5 × 3H, *s*, 18-, 19-, 28-, 29-, 30-Me), 1.11, 1.15, 1.18 (3 × 3H, *s*, 21-, 26-, 27-Me), 3.64 (1H, *m*, H-24), 3.49 (2H, *s*, CH_2-31). ^{13}C NMR (50 MHz, $CDCl_3$): δ 218.2 (C-3), 86.5 (C-24), 86.3 (C-20), 70.2 (C-31), 55.3 (C-5), 50.2 (C-9), 50.0 (C-14), 49.7 (C-17), 47.4 (C-4), 42.9 (C-13), 40.3 (C-8), 39.9 (C-1), 36.8 (C-10), 34.7 (C-22), 34.6 (C-7), 34.1 (C-2), 31.4 (C-15), 29.7 (C-25), 27.8 (C-28), 27.2 (C-27), 27.0 (C-16), 26.7 (C-26), 26.3 (C-23), 25.8 (C-12), 24.0 (C-21), 22.3 (C-11), 21.0 (C-29), 19.6 (C-6), 16.3 (C-30), 16.1 (C-18), 15.2 (C-19).

6α,11β-Diacetoxygedunin (**5**). Solid; Mp 187–192°. TLC: $R_f = 0.35$. UV λ_{max}^{MeOH} nm (log ε): 238 (3.83). $[\alpha]_D^{25} = +87°$ ($CHCl_3$; *c* 0.17). D/CIMS *m/z* (rel. int.): 616 $[M + NH_4]^+$(100). ^1H NMR as ref. [18]. ^{13}C NMR (50 MHz, $CDCl_3$): δ 203.3 (C-3), 170.0, 169.8, 169.6 (3 × O<u>C</u>OMe), 166.5 (C-16), 154.0 (C-1), 143.4 (C-21), 141.3 (C-23), 127.1 (C-2), 119.7 (C-20), 109.7 (C-22), 77.8 (C-17), 73.5* (C-7), 69.4* (C-6), 68.6 (C-14), 66.0* (C-11), 55.1 (C-15), 48.9 (C-9), 44.6 (C-4), 42.7 (C-8), 41.9 (C-5), 41.1 (C-10), 37.7 (C-13), 36.1 (C-12), 31.7, 23.9, 21.5, 21.2, 20.9, 20.5, 20.0, 17.0, (8 × Me). *Signals interchangeable.

1,8b-Dihydroxy-6,8-dimethoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydrocyclopenta[b]-benzofuran-2(1H)-carboxylate (**6**). Light yellow solid; mp 87–90°. TLC: $R_f = 0.18$. UV λ_{max}^{MeOH} nm (log ε): 229 (4.1), 285 (3.72). $[\alpha]_D^{25} = -35°$ ($CHCl_3$; *c* 0.5). TSPMS *m/z* (rel. int.): 493 $[M + H]^+$, 475 (20), 313 (65). ^1H and ^{13}C NMR as ref. [9].

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