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Ponapensin, a cyclopenta[*bc*]benzopyran with potent NF-κB inhibitory activity from *Aglaia ponapensis*

Angela A. Salim,[a] Alison D. Pawlus,[a,b] Hee-Byung Chai,[a] Norman R. Farnsworth,[b] A. Douglas Kinghorn[a] and Esperanza J. Carcache-Blanco[a,c,*]

[a]*Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
[b]*Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA*
[c]*Division of Pharmacy Practice and Administration, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*

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Abstract—Two new compounds, a cyclopenta[*bc*]benzopyran, ponapensin (**1**), and an aglaialactone, 5,6-desmethylenedioxy-5-methoxy-aglalactone (**2**), together with nine known compounds were isolated from the $CHCl_3$ soluble extract of the leaves and twigs of *Aglaia ponapensis*. Their structures were established by spectroscopic data interpretation. Ponapensin (**1**) exhibited significant NF-κB inhibitory activity in an Elisa assay, and was found to be more potent than the positive control rocaglamide. All of the compounds isolated were also tested in a panel of human cancer cell lines, with the known sterol *E*-volkendousin (**3**) and methyl rocaglate (aglafoline) found to be the only active substances.

The genus *Aglaia* Lour. (Meliaceae) consists of about 130 species, which are distributed mainly in the Indo-Malayan region, southern mainland China, and the Pacific Islands.[1] Since rocaglamide, the first cyclopenta[*b*]benzofuran, was isolated as a constituent of *Aglaia elliptifolia*, and was found to be active in an in vivo P388 model,[2] the phytochemical investigation of the genus *Aglaia* has led to the isolation of many related compounds. To date, about 60 naturally occurring cyclopenta[*b*]benzofuran type compounds, many of which exhibit insecticidal and antiproliferative activities, have been isolated from over 30 *Aglaia* species.[3,4] The cyclopenta[*b*]benzofurans and two structurally related groups, the cyclopenta[*bc*]benzopyrans and benzo[*b*]oxepines, are considered characteristic secondary metabolites of the genus *Aglaia*, because they have been isolated only from this taxon.[3] The collective name "flavagline" has been proposed for these compounds because their mutual biogenetic origin has been postulated to be a flavonoid nucleus linked to a cinnamic acid moiety.[5,6] Among the flavaglines, many members of the cyclopenta[*b*]benzofuran-type compounds have shown pronounced inhibitory activity against cancer cell lines at nanomolar concentrations, while the cyclopenta[*bc*]benzopyrans and benzo[*b*]oxepines evaluated so far were not active.[4] Cyclopenta[*b*]benzofurans have also been shown to inhibit TNF-α or PMA-induced NF-κB activity in different mouse and human T-lymphocyte cell lines.[7] NF-κB has been shown to be crucial for inducing genes involved in inflammation and in a wide range of diseases originating from chronic activation of the immune system, including colon cancer.[8] Thus, NF-κB may play a key role in regulating the expression of pro-inflammatory and/or apoptotic genes in cancer, making it an attractive target for therapeutic intervention.

In a preliminary study of the stems of *Aglaia ponapensis* Kaneh. (syn. *A. mariannensis* Merr.) carried out in our laboratories, it was discovered that this species is a good source of methyl rocaglate (aglafoline).[9] Methyl rocaglate is the most frequently tested compound among the cyclopenta[*b*]benzofurans, and overall it has been found to exhibit slightly more potent inhibitory activity compared to rocaglamide against the several cancer cell lines in which it has been examined.[4] Therefore, the large-

Keywords: Ponapensin; Cyclopenta[*bc*]benzopyran; NF-κB; *Aglaia ponapensis*.
* Corresponding author. Tel.: +1 614 247 7815; fax: +1 614 292 1335; e-mail: carcache-de-blanco.1@osu.edu

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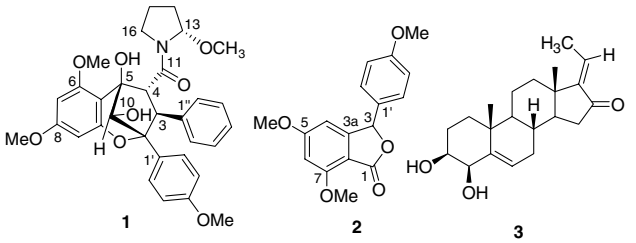
scale isolation of methyl rocaglate was carried out from the leaves and stems of *A. ponapensis* (8 kg) collected in Ponape Island, the Federated States of Micronesia, so that this compound could be utilized for chemical transformation and biological studies.[10] The air-dried plant material of *A. ponapensis* was extracted with methanol, and partitioned in turn with *n*-hexane, $CHCl_3$, and EtOAc. The $CHCl_3$-soluble fraction was subjected to fractionation using silica gel, Diaion HP-20, Sephadex LH-20, and reversed-phase HPLC to afford two new (**1** and **2**) and nine known compounds.[11] The new compound **1**, ponapensin, was found to be a potent inhibitor of NF-κB in an Elisa assay carried out in our laboratory. In addition, all compounds isolated were also tested against a small panel of cancer cell lines.



Compound **1** was obtained as yellow oil, $[\alpha]_D^{22}$ −167 (*c* 0.4, $CHCl_3$) and gave a sodiated molecular ion peak at *m*/*z* 584.2266 (calcd for $C_{32}H_{35}NO_8Na$, 584.2260) in the HRESIMS.[12] The ^1H NMR spectrum showed signals for three aromatic rings: a monosubstituted benzene ring at δ_H 7.18 (2H, d, J = 6.8 Hz, H-2″, 6″) and 6.96 (3H, m, H-3″–H-5″), a *para*-disubstituted benzene ring at δ_H 7.40 (2H, d, J = 8.9 Hz, H-2′, 6′) and 6.61 (2H, d, J = 8.9 Hz, H-3′, 5′), and two *meta*-coupled aromatic protons at δ_H 6.12 (1H, d, J = 2.2 Hz, H-7) and 6.03 (1H, d, J = 2.2 Hz, H-9). In addition, signals belonging to three methoxy groups [δ_H 3.79 (3H, s, OMe-6), 3.73 (3H, s, OMe-8) and 3.67 (3H, s, OMe-4′)], an oxygenated methine [δ_H 4.62 (1H, s, H-10)], and two vicinal methines [δ_H 4.40 (1H, d, J = 9.4 Hz, H-3) and 4.36 (1H, d, J = 9.4 Hz, H-4)] were observed, suggesting the presence of a cyclopenta[*bc*]benzopyran moiety.[6] Analysis of the remaining signals by DQF-COSY revealed the presence of a pyrrolidine ring. Consistent with the ^1H NMR spectrum of compound **1**, its ^{13}C NMR spectrum also displayed signals characteristic of a cyclopenta[*bc*]benzopyran skeleton. The signal for C-13 at δ_C 89.6 suggested the presence of an oxygen atom next to this methine carbon, and an HMBC cross-peak between δ_H 5.80 (H-13) and δ_C 54.3 (OMe-13) confirmed the location of the methoxy group at C-13. HMBC correlations from δ_H 4.40 (H-3) to δ_C 131.4 (C-2″, 6″) and 90.0 (C-2), and from δ_H 4.36 (H-4) to δ_C 172.4 (C-11), 143.1 (C-1″), 107.3 (C-5a), and 83.1 (C-5) established the locations of the aromatic ring and the pyrrolidine ring at C-3 and C-4, respectively. The relative configuration of **1** was determined from analysis of the ^1H NMR coupling constant and the 2D NOESY data (Fig. 1). The configuration at C-3 and C-4 was determined as H-3α and H-4β, based on the

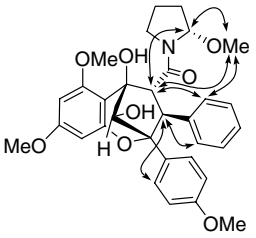


Figure 1. Selected 2D NOE correlations for ponapensin (**1**).

$^3J_{(H\text{-}3,H\text{-}4)}$ coupling constant (J = 9.4 Hz).[13] In the 2D NOESY spectrum, a correlation between H-10 and H-4 was not observed, suggesting an exo relationship between the two protons. The configuration at C-13 was *S*, as a result of the NOE correlations observed between H-13/H-4, H-13/OMe-13, OMe-13/H-4, and OMe-13/H2″,6″, and examination using Dreiding models. The Insight II molecular modeling program was used to generate a 3D model of **1** (Fig. 2); the optimized model showed that OMe-13 was placed in the shielding zone of the monosubstituted benzene ring, which is in agreement with the NOEs observed and the upfield proton chemical shift of OMe-13 (δ 2.80). Accordingly, compound **1** was assigned as (−)-*rel*-(2*R*,3*S*,4*R*,5*R*,10*S*,2′*S*)-1-[2,3,4,5,-tetrahydro-5,10-dihydroxy-2-(4-methoxyphenyl)-6,8-dimethoxy-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-methoxypyrrolidine, to which we have given the trivial name, ponapensin.

Compound **2** was isolated as a white amorphous powder, $[\alpha]_D^{22}$ −13 (*c* 1.1, $CHCl_3$). Its HRESIMS exhibited a sodiated molecular ion peak at *m*/*z* 323.0895, indicating an elemental formula of $C_{17}H_{16}O_5Na$ (calcd 323.0890).[14] The ^1H NMR spectrum showed signals for two aromatic rings, constituted by two *meta*-coupled aromatic protons at δ_H 6.25 (1H, d, J = 0.9 Hz, H-4) and 6.43 (1H, d, J = 1.4 Hz, H-6), and a characteristic AA′BB′ system of a *p*-disubstituted benzene ring at δ_H 7.17 (2H, d, J = 8.5 Hz, H-2′,6′) and 6.88 (2H, d, J = 8.5 Hz, H-3′,5′). In addition, two singlets characteristic of OMe groups [δ_H 3.96 (3H, s, OMe-7), 3.79 (6H, s, OMe-5, OMe-4′)] and a downfield methine signal at δ_H 6.17 (H-3) were observed. The ^{13}C NMR spectrum displayed the signals for a tetrasubstituted and a disubstituted benzene ring, and three methoxy carbons,

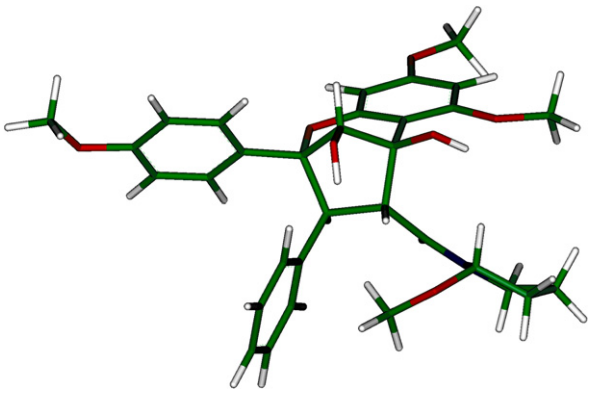


Figure 2. 3D structure of ponapensin (**1**).

consistent with the ^{1}H NMR data. Furthermore, an unsaturated carbonyl (δ_C 168.4, C-1) and an oxygenated methine carbon (δ_C 81.4, C-3) were also observed, suggesting the presence of a benzofuranone moiety. In the HMBC spectrum, correlations were observed from H-3 to C-1, C-3a, C-4, C-1′, and C-2′,6′, confirming the position of the lactonic proton as *ortho* relative to the ring fusion position C-3a. The structure of **2** is related to that of aglalactone,[15] except for the absence of a 5,6-methylenedioxy group and the presence of an extra methoxy group in C-5. Accordingly, this new compound was assigned as 5,7-dimethoxy-3-(4-methoxyphenyl)-1,3-dihydrobenzo[*c*]-furan-1-one, or 5,6-desmethylenedioxy-5-methoxy-aglalactone.

Nine known compounds, a cyclopenta[*b*]benzofuran (methyl rocaglate,[16]) four cyclopenta[*bc*]benzopyrans (4-*epi*-aglain A,[17] aglain B,[18] 10-*O*-acetylaglain B,[17] and aglain C[18]), and four pregnane steroids [(*E*)-volkendousin (**3**),[19] (*Z*)-volkendousin,[19] 2β,3β-dihydroxy-5-pregn-17(20)-(*E*)-en-16-one,[20] and 2β,3β-dihydroxy-5-pregn-17(20)-(*Z*)-en-16-one[20]] were also isolated from *A. ponapensis*. All of these compounds, except methyl rocaglate, are reported for the first time from this species. The occurrence of pregnane steroids as isolated in this study is quite rare in the plant kingdom, and such compounds have only been isolated from two species in the Meliaceae family (*Melia volkensii* and *Aglaia grandis*).[19,20]

In an enzyme-based Elisa assay NF-κB assay,[21,22] ponapensin (**1**) showed a potent NF-κB inhibitory activity with an IC_{50} of 0.06 μM, while rocaglamide (positive control) and methyl rocaglate exhibited IC_{50} values of 2.0 and 2.3 μM, respectively. The NF-κB inhibitory activity of **2** (IC_{50} = 1.9 μM) was comparable to that of the positive control. The other cyclopenta[*bc*]benzopyran-type compounds isolated in this study (4-*epi*-aglain A, aglain B, 10-*O*-acetylaglain B, and aglain C) were not active in the NF-κB assay (IC_{50} > 5 μM). Only one cyclopenta[*bc*]benzopyran-type compound has been tested previously for NF-κB inhibitory activity, using Jurkat T and HeLa cells, and was found to be inactive.[7] It is interesting that a change in the pyrrolidine side chain of the cyclopenta[*bc*]benzopyran-type compounds, from a methylbutanoylamino group as found in aglain C, to a methoxy group as found in ponapensin, drastically enhanced the NF-κB inhibitory activity. This finding may be significant for the structure–activity relationship (SAR) study of this type of compound. Further biological studies need to be performed to uncover the specific mechanism of NF-κB inhibition of the potent compound **1**, such as finding whether the IκB kinase complex is a target.

All compounds isolated from this study, except methyl rocaglate, were also tested in a panel of cancer cell lines.[23] Only one compound, *E*-volkendousin (**3**), was found to be cytotoxic, with ED_{50} values of 4.6, 4.7, and 4.2 μg/mL against Lu1, LNCaP, and MCF-7 cell lines, respectively. Methyl rocaglate is a known cytotoxic agent, and has been tested previously in a panel of cancer cell lines in our laboratory.[24]

In conclusion, two new (**1** and **2**) and nine known compounds have been isolated from the stems and leaves of *A. ponapensis* in the present study. The relative configuration of new compound **1**, ponapensin, has been confirmed by 2D NOESY data, observation using Dreiding models, and molecular modeling.

Acknowledgments

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10. The leaves and stem of *A. ponapensis* Kaneh. (Meliaceae) were collected in rainforest at 600 ft. elevation on Mt. Poaipoai, Ponape Island, one of the Eastern Caroline Islands, the Federated States of Micronesia. The plant was collected by Y. Sagawa, University of Hawaii, Honolulu, HI, and identified by S. F. Glassman. A voucher specimen (Accession No. 2264217) has been deposited at the Economic Botany Laboratory, USDA, Beltsville, MD, USA.
11. The dried and milled leaves and stems of A. ponapensis (8 kg) were extracted using MeOH (3 × 28 L) at rt, for 48 h each. The combined extracts were evaporated in vacuo, and water was added to give a 10% methanolic extract (4 L), followed by subsequent partition with hexane (3 × 4 L), $CHCl_3$ (3 × 4 L), and EtOAc (3 × 4 L). The $CHCl_3$-soluble extract (115 g) was chromatographed over

a silica gel column (12 × 33 cm, 70–230 mesh) and eluted with $CHCl_3$/MeOH (99:1 → 9:1). Fractions eluting in 5–7.5% MeOH were combined (20 g) and further chromatographed over a silica gel column (7.5 × 43 cm, 230–400 mesh) using mixtures of hexane/EtOAc/MeOH (10:10:0.1 → 10:10:2) as solvents to give 11 sub-fractions (Ap01–Ap11). Sub-fraction Ap07 (1.2 g, eluted with hexane/EtOAc/MeOH, 10:10:1) was chromatographed over Diaion HP-20 gel (eluted with 90% MeOH) to remove the chlorophylls, followed by Sephadex LH-20 gel (2.5 × 75 cm, in MeOH) to afford pure methyl rocaglate (270 mg). Sub-fraction Ap08 (0.9 g, eluted with hexane/EtOAc/MeOH, 10:10:1.2) was processed similarly to the previous fraction to afford an additional quantity of methyl rocaglate (30 mg) and 5,6-desmethylenedioxy-5-methoxyaglalactone (**2**) (15 mg). Sub-fraction Ap09 (2 g, eluted with hexane/EtOAc/MeOH, 10:10:1.4), was chromatographed sequentially on Diaion HP-20, Sephadex LH-20, and C_{18} RP-HPLC (MeOH–H_2O, 50 → 70% in 20 min, then isocratic at 70%) to furnish ponapensin (**1**) (5 mg, t_R 29.4 min), 2β,3β-dihydroxy-5-pregn-17(20)-(*Z*)-en-16-one (5 mg, t_R 32.4 min), (*Z*)-volkendousin (1 mg, t_R 34.5 min), 2β,3β-dihydroxy-5-pregn-17(20)-(*E*)-en-16-one (2 mg, t_R 39.0 min), and (*E*)-volkendousin (**3**) (1 mg, t_R 41.5 min). Aglain C (300 mg) precipitated from sub-fraction Ap11 (4 g, eluted with hexane/EtOAc/MeOH, 10:10:1.8) as a white amorphous powder, and the filtrate was treated in a similar fashion to sub-fraction Ap09 (C_{18} RP-HPLC, MeOH–H_2O, isocratic at 60% for 15 min, 60 → 70% in 5 min, then isocratic at 70%) to afford 10-*O*-acetylaglain B (40 mg, t_R 41.8 min), aglain B (21 mg, t_R 45.9 min), and 4-*epi*-aglain A (65 mg, t_R 49.5 min).

12. Yellow oil, $[\alpha]_D^{22}$ −167 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 224 (3.84) nm; IR (film) ν_{max} 3474, 2936, 1618, 1590, 1518, 1437, 1251, 1214, 1148, 1082 cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4, calibrated at δ_H 3.30) δ 7.40 (2H, d, *J* = 8.9 Hz, H-2′,6′), 7.18 (2H, d, *J* = 6.8 Hz, H-2″,6″), 6.96 (3H, m, H-3″–H-5″), 6.61 (2H, d, *J* = 8.9 Hz, H-3′,5′), 6.12 (1 H, d, *J* = 2.2 Hz, H-7), 6.03 (1H, d, *J* = 2.2 Hz, H-9), 5.80 (1H, br d, *J* = 3.3 Hz, H-13), 4.62 (1H, s, H-10), 4.40 (1H, d, *J* = 9.4 Hz, H-3), 4.46 (1H, d, *J* = 9.4 Hz, H-4), 3.79 (3H, s, OMe-6), 3.73 (3H, s, OMe-8), 3.67 (3H, s, OMe-4′), 3.34 (2H, m, H_2-16, overlap with MeOH-d_4), 2.80 (3H, s, OMe-13), 2.02 (1H, m, H-14a), 1.90 (2H, m, H_2-15), 1.88 (1H, m, H-14b); ^{13}C NMR (100 MHz, MeOH-d_4, calibrated at δ_C 49.0) δ 172.4 (C-11), 162.7 (C-8), 160.0 (C-4′), 159.8 (C-6), 155.0 (C-1a), 143.1 (C-1″), 132.0 (C-1′), 131.7 (2C, C-2′,6′), 131.4 (2C, C-2″,6″), 128.6, (2C, C-3″,5″), 127.0 (C-4″), 113.4 (2C, C-3′/5′), 107.3 (C-5a), 95.2 (C-9), 92.8 (C-7), 90.0 (C-2), 89.6 (C-13), 83.1 (C-5), 80.9 (C-10), 63.6 (C-4), 58.5 (C-3), 56.5 (OCH_3-6), 55.8 (OCH_3-8), 55.5 (OCH_3-4′), 54.3 (OCH_3-13), 46.3 (C-16), 32.7 (C-14), 21.7 (C-15); HRESIMS *m*/*z* 584.2266 $[M+Na]^+$ (calcd for $C_{32}H_{35}NO_6Na$, 584.2260).
13. Previous studies of other cyclopenta[*bc*]benzopyran-type compounds have shown that $^3J_{(H\text{-}3,H\text{-}4)}$ of 5–6 Hz is compatible with the H-3β, H-4α configuration, while the vicinal coupling constant of 9–10 Hz is compatible with the H-3α, H-4β configuration.[3,6]
14. White amorphous powder; $[\alpha]_D^{22}$ −13 (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 257 (3.99), 220 (4.37), 208 (4.26) nm; IR (film) ν_{max} 1755, 1612, 1514, 1462, 1330, 1259, 1157, 1058 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 7.17 (2H, d, *J* = 8.5 Hz, H-2′,6′), 6.88 (2H, d, *J* = 8.5 Hz, H-3′,5′), 6.43 (1H, d, *J* = 1.4 Hz, H-6), 6.25 (1H, d, *J* = 0.9 Hz, H-4), 6.17 (1H, s, H-3), 3.96 (3H, s, OMe-7), 3.79 (6H, s, OMe-5, OMe-4′); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 168.4 (C-1), 166.8 (C-5), 160.2 (C-4′), 159.4 (C-7), 154.9 (C-3a), 128.7 (2C, C-2′,6′), 128.6 (C-1′), 114.2 (2C, C-3′,5′), 106.6 (C-7a), 99.0 (C-6), 98.3 (C-4), 81.4 (C-3), 56.0 (OCH_3-7), 55.9 (OCH_3-5 or OCH_3-4′), 55.3 (OCH_3-5 or OCH_3-4′); HRESIMS *m*/*z* 323.0895 $[M+Na]^+$ (calcd for $C_{17}H_{16}O_5Na$, 323.0890).
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22. The NF-κB assay was carried out according to Ref. 21. An ELISA (EZ-Detect™ Transcription Factor Assay System) available from Pierce Biotechnology (Rockford, IL) was used to assess the ability of all compounds to interfere with the specific binding between the biotinylated-consensus sequence for the respective factor and the active form of NF-κB transcription factor. Nuclear extract of HeLa cells (purchased from the American Type Culture Collection, ATCC # CCL-2) treated with TNF-α and test compound was used for evaluation of specific binding. The detection of NF-κB activity was based on the measurement of a chemiluminescent signal in the plate reader Fluostar Optima from BMG Labtechnologies Gmbh, Inc. (Durham, NC). TNF-α stimulated nuclear extract was used as a control in the chemiluminescent assay. Rocaglamide was used as a positive control in the NF-κB assay.
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