Icosandrin, a novel peltogynoid from the fruits of *Phytolacca icosandra* (Phytolaccaceae)

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Besides the known compounds (\(\pm\)) 3,3-bis-demethylpinoresinol (2), americanol A (3), spergulagenic acid (4), epi-acetylaaleuritolic acid (5), 6\(^{\beta}\)-palmityl-\(\alpha\)-spinateryl-D-glucoside (6a) and 6\(^{\beta}\)-palmityl-\(\delta\)-stigmastenyl-D-glucoside (6b), a novel peltogynoid (1) named icosandrin was obtained from the dried fruits of *Phytolacca icosandra*. This new compound was characterised by 1D-/2D-NMR, UV, IR and HR-MS techniques as 11\(^\beta\)-methoxy-6,7-methylenedioxy-[2]benzopyran-[4,3-\(b\)]-[1]-benzopyran-4-one. Toxicity of 1 was assessed through the Brine Shrimp Lethality Assay. Lignan 2 is reported for the first time in Phytolaccaceae family.

**Keywords:** Phytolaccaceae; *Phytolacca icosandra*; peltogynoids; lignans; BSLA

1. **Introduction**

*Phytolacca icosandra* (Phytolaccaceae) is a medium-high shrub, native of tropical regions of America (USA to Perú) and distributed also in Australia, New Zealand and South Africa regions (Steinmann 1997; Rzedowski & Calderón de Rzedowski 2000). Plants belonging to this genus have been used in folk medicine around the world, for the treatment of several affections such as oedema, rheumatism and dermatitis (Jolliffe 1982; Williams et al. 2002; Ravikiran et al. 2011) and also as molluscicidal in schistosomiasis prevention and control (Lemma 1970; Lambert et al. 1985). Several studies on *P. icosandra* for testing its antisecretory, anthelmintic, ovicidal and larvicidal activity have been reported (Santos-López et al. 2010; Hernández-Villegas et al. 2011, 2012). The chemistry of Phytolaccaceae is fairly wide and comprises a variety of secondary metabolites such as triterpenoidal saponins, flavonoids and lignans (Williams et al 2002). Previous phytochemical analysis of *P. icosandra* has lead to the isolation of serjanic and spergulagenic acid saponins (Treyvaud et al. 2000; Galarraga et al. 2014).

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Peltogynoids (also known as cyclic homoflavonoids) are a type of natural products represented by a peltogynane skeleton, which is a rare type of modified flavonoid with specific cyclisation between 3-OH and C-2′ of ring B; they can be formally related to anthocyanidins, flavan-3,4-diols, flavonols and dihydro-flavonols (Gottlieb & Rego de Souza 1972; Choudhary et al. 2001). The present article deals with the isolation and characterisation of a new natural peltogynane (1), and its chemotaxonomic implications. We also report on the in vitro toxicity of 1 against brine shrimps (Artemia salina).

2. Results and discussion

2.1. Structure elucidation of isolated compounds

Compound 1 was isolated as a pale yellow solid m.p. <300°C. The ESI mass spectra displayed a pseudo molecular-ion peak at m/z 325.07 [M + H]+ suggesting the molecular formula C_{18}H_{12}O_{6}, in agreement with thirteen degrees of unsaturation. The structure was first suggested to possess a flavone skeleton, based on the physicochemical properties and chromatography performance. IR absorptions at 1625 and 1495 (C–C), 1634 (C–O) and 1255, 1025 (C–O–C) cm⁻¹, along with UV maximal absorptions at 332 and 237 nm, confirmed that the compound was included in the flavone series.

{^{13}C} NMR and DEPT spectra of 1 displayed resonances for one carbonyl group (δ_C 171.0, C-4), fourteen sp² olefinic or aromatic carbons (δ_C 97.8–152.7), one acetalic carbon (δ_C 98.5, C-11), one dioxymethylene (δ_C 102.5) and one methoxyl group (δ_C 56.1). The {^1}H NMR spectrum revealed the presence of two independent six carbon aromatic systems, the first characterising a tetra-substituted ring whose signals were located at δ_H 6.98 (1H, s, H-8) and 7.63 (1H, s, H-5), and the second identifying a 1,2-di-substituted ring with signals at δ_H 7.41 (1H, dd, J = 1.2 and 7.2 Hz, H-3′), 7.94 (1H, dd, J = 1.2 and 7.2 Hz, H-6′), 7.56 (1H, td, J = 1.3 and 7.5 Hz, H-5′) and 7.59 (1H, td, J = 1.3 and 7.5 Hz, H-4′), typical to the orto-coupled C-3′, C-4′, C-5′ and C-6′ protons. Methylenedioxy group was supported by the presence of a two-proton broad singlet at δ_H 6.12 and IR absorption at 930 cm⁻¹. This group was located at the tetra-substituted aromatic system by the HMBC cross peaks between the −O−CH₂−O− protons and two sp² quaternary carbons at δ_C 146.0 (C-6) and 152.7 (C-7), which also showed interactions with the aromatic hydrogens H-5 and H-8, respectively.

HMBC interaction between one of the aromatic hydrogens of the 1,2-di-substituted ring at δ_H 7.41 (H-3′) and the acetalic carbon at δ_C 98.5 (C-11), confirmed the pyrano-type structure representative of peltogynoids. Methoxyl group at δ_H 3.64 was also located at the C-11 position by a HMBC cross peak with the signal at δ_C 98.5. All the remaining assignments of {^1}H and {^{13}}C NMR data were achieved by HMQC and HMBC experiments. Stereochemistry at C-11 centre remained unknown due to the absence of vicinal protons with which H-11 may form a dihedral angle. From the above-described spectral evidence, compound 1 was identified as 11j-methoxy-6,7-methylenedioxy-[2]benzopyrano-[4,3-b][1]benzopyran-4-one (Figure 1). The presence of the C-11 acetalic carbon, more commonly found in rotenoids at C-6 position (Crombie & Whiting 1998), has been seen so far only in the peltogynoid fasciculiferin, isolated from Acacia fasciculifera (van Heerden et al. 1979). Toxicity of 1 was assayed in the Brine Shrimp Lethality Assay (Meyer et al. 1982); it was found that icosandrin exhibited a toxic effect over A. salina (IC_{50} = 158 ± 36 µg/mL).

2.2. Chemotaxonomic significance

Despite Phytolacca genus is characterised for biosynthesise mainly triterpenoidal saponins, some related peltogynane-type compounds, such as flavonoids, have been obtained from Phytolacca americana (Bylka & Matlawska 2001), Phytolacca thyrsiflora (Haraguchi et al. 1988) and
Phytolacca dioica (Soliman & Sobieh 1999). On the other hand, peltogynanes are metabolites with some restricted distribution in nature and to the best of our knowledge, they have been isolated mainly from genus belonging to the Leguminosae family such as Acacia (Ahmadu et al. 2010), Caesalpinia (McPherson et al. 1983), Colophospermum (Malan et al. 1990), Derris (Koysomboon et al. 2006), Distemonanthus (Malan & Roux 1979), Goniorrhachis (Gottlieb & Rego de Souza 1972), Peltogyne (de Almeida et al. 1974) and Trachylobium (Ferreira et al. 1974). The other sources outside of Leguminosae family are Cassine (Celastraceae) (Drewes & Mashimbye 1993), Iris (Iridaceae) (Choudhary et al. 2001), Macaranga and Croton (Euphorbiaceae) (Li et al. 2009; Zou et al. 2010) and Ophioglossum (Ophioglossaceae) (Lin et al. 2005). As observed, peltogynoids are limited to a few families, making the presence of 1 in Phytolaccaceae a very uncommon feature that could be used in chemotaxonomic studies for P. icosandra.

The presence of lignans in Phytolacca is also limited, with reports only for P. americana (Sick Woo et al. 1980) and P. thyrsiflora (Haraguchi et al. 1988). Compound 2 has been previously isolated from Joannesia princeps (Euphorbiaceae) (Waibel et al. 2003), and this is the first report for Phytolaccaceae. Compounds 3, 6a and 6b were found in P. americana (Woo 1974; Sick Woo et al. 1980), whilst 5 in P. acinosa (Razdan et al. 1982). Compound 4 has only been characterised as the aglycone of several saponins from P. icosandra (Treyvauld et al. 2000).

3. Experimental

3.1. General procedures

Melting points were determined with a Fisher-Johns apparatus and were not corrected. Optical rotation was measured in Rudolph Research Autopol™ III equipment. UV spectra were obtained in chloroform using a Perkin-Elmer, Lambda-3B spectrophotometer. IR spectra were recorded on a Perkin-Elmer FT-1725X spectrophotometer using KBr pellets. 1D and 2D NMR spectra were acquired with a Bruker-Avance DRX-400 instrument, using CDCl3 as solvent. ESI-MS was run on a TSQ QUANTUM, Ultra AM, Thermo Scientific Spectrophotometer. The HR-MS analysis was conducted in a JEOL JMS-AX505WA spectrometer with direct inlet and dual approach mass analyser, using electron impact (EI) method. TLC was carried out on 0.25 mm layers of silica gel PF254 (Merck, Kenilworth, NJ, USA).

3.2. Plant material

P. icosandra fruits were collected in Mucuhies-Gavidia, Municipio Rangel, Estado Mérida-Venezuela, in August 2008, and identified by Ing. For. Juan Carmona Arzola, Universidad de
Los Andes (Mérida-Venezuela). A voucher specimen (J. M. Amaro et al., No. 2322) was deposited in the MERF herbarium, Faculty of Pharmacy, ULA.

3.3. Extraction and isolation

Air-dried and powdered fruits of *P. icosandra* (1.1 kg) were exhaustively extracted at room temperature with aqueous MeOH (70%, v/v) in a Soxhlet for 24 h. After vacuum evaporation of the solvent, the crude extract (150 g) was pre-absorbed on normal-phase silica gel and submitted to a chromatographic process (CC), using Hex/CH$_2$Cl$_2$ (0% up to 100%), Hex/EtOAc (30% up to 100%) and CH$_2$Cl$_2$/MeOH (20% up to 100%) mixture solvents, to afford 15 fractions (A-O). A white impure solid was filtered from fraction ‘D’ (4.1 g, Hex/CH$_2$Cl$_2$ 4:1) which was subsequently washed and recrystallised from EtOH to afford 5 (40.1 mg). Compound 1 (14.2 mg), was obtained from fraction ‘G’ (1.15 g, Hex/EtOAc 3:2), subfraction G$_2$ (85.1 mg), chromatographed on a silica gel column (Hex/EtOAc 30%) and finally purified by preparative thin-layer chromatography, using Hex/EtOAc 35% as eluent. A portion of fraction ‘I’ (2.53 g, EtOAc 100%), was further fractioned and purified by several CC on silica gel to afford 2 (16.1 mg) and 4 (20.5 mg) from subfraction I$_1$ (Hex/EtOAc 55%), 3 (14.7 mg) from subfraction I$_2$ (Hex/EtOAc 60%) and 6a + 6b (18.5 mg) from subfraction I$_3$ (Hex/EtOAc 70%).

3.4. Identification of known compounds

Known compounds were identified by comparison of their physical constants and NMR spectroscopic data with those reported in the literature (Chakrabarty et al. 1968; Woo 1974; Fukuyama et al. 1992; Razdan et al. 1982; Waibel et al. 2003).

3.5. Icosandrin (1)

Pale yellow powder, m.p.: < 300°C; [α]$_D^{23}$ + 116.7° (c 0.0003 w/v%, CHCl$_3$); R$_f$: 0.43 (Hex/ EtOAc, 1:1); UV (CHCl$_3$) $\lambda_{max}$ (log $\varepsilon$): 237 (3.9), 332 (0.8) nm. IR (KBr): 2921, 1634, 1625, 1462, 1255, 1025 and 930 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm): 3.64 (3H, s, 11-OCH$_3$), 6.08 (1H, s, H-11), 6.12 (2H, s, −O−CH$_2$−O−), 6.98 (1H, s, H-8), 7.41 (1H, dd, J = 1.2, 7.2 Hz, H-3'), 7.56 (1H, td, J = 1.3, 7.5 Hz, H-5'), 7.59 (1H, td, J = 1.3, 7.5 Hz, H-4'), 7.63 (1H, s, H-5) and 7.94 (1H, dd, J = 1.2, 7.2 Hz, H-6'). $^{13}$C NMR (100 MHz, CDCl$_3$) δ (ppm): 56.1 (11-OCH$_3$), 97.8 (C-8), 98.5 (C-11), 102.4 (C-5), 102.5 (−O−CH$_2$−O−), 119.4 (C-10), 121.6 (C-6'), 123.8 (C-1'), 126.3 (C-3'), 129.9 (C-5'), 130.9 (C-2'), 131.0 (C-4'), 133.7 (C-3), 145.9 (C-2), 146.0 (C-6), 152.1 (C-9), 152.7 (C-7) and 171.0 (C-4). ESI-MS m/z (Rel. Int. %): 325.07 [M$^+$ + H] (100); 293.02 [M$^+$ + H−CH$_3$OH] (11); HR-MS m/z 324.0637 [M$^+$] (calcd. for C$_{18}$H$_{12}$O$_6$, 324.0634).

3.6. Brine shrimp lethality assay

The assay was performed as described previously by Meyer et al. (1982) with some minor modifications. Brine shrimp eggs (Gulf Breeze®) were hatched in artificial seawater prepared with commercial salt mixture (Instant Ocean®), illuminated and oxygenated with an aquarium pump. After 48 h incubation at 27°C, 10 shrimps were transferred with a Pasteur pipette to three sample vials for each of three doses (100, 50 and 10 μg/mL) for a total of nine vials. The sample was prepared by dissolving the compound (3 mg) in CHCl$_3$ (5 mL) and transferring the solution to each vial (833, 417 or 83 μL solution for 100, 50 or 10 ppm doses) followed by high vacuum for 1 h. After the solvent was evaporated, the compound was redissolved in 20 μL of Tween 80® and 5 mL of artificial sea water were added to achieve the correct concentration. Survivors were counted and the percent deaths at each dose and control were determined. Tween 80® at this
concentration did not affect this bioassay. The IC$_{50}$ and 95% confidence intervals were calculated from 24 h counts, using the Probit analysis method (Finney 1971).

**Supplementary material**

Supplementary material relating to this article is available online

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

Ahmadu A, Abdulkarim A, Grougnet R, Myrianthopoulos V, Tillequin F, Magiatis P, Skalsounis AL. 2010. Two new peltogynoids from *Acacia nilotica* Delile with kinase inhibitory activity. Planta Med. 76:458–460. doi:10.1055/s-0029-1186226.

Bylka W, Matlawska I. 2001. Flavonoids and free phenolic acids from *Phytolacca americana* L. leaves. Acta Pol Pharm. 58:69–72.

Chakrabarty P, Mukherjee DK, Barua AK. 1968. The structure and stereochemistry of spargulagenic acid. Tetrahedron. 24:1107–1111. doi:10.1016/0040-4020(68)80059-7.

Choudhary MI, Nur-e-alam M, Akhtar F, Ahmad S, Baig I, Ondögnii P, Gombosuren-gin P, Rahman. 2001. Five new peltogynoids from underground parts of *Iris bunage*: a mongolian medicinal plant. Chem Pharm Bull. 49:1295–1298. doi:10.1248/cpb.49.1295.

Crombie L, Whiting DA. 1998. Biosynthesis in the rotenoid group of natural products: Application of isotope methodology. Phytochemistry. 49:1479–1507. doi:10.1016/S0031-9422(98)00178-2.

de Almeida EM, Gottlieb OR, Rêgo de sousa JR, Teixeira MA. 1974. New peltogynoids from three *Peltogyne* species. Phytochemistry. 13:1225–1228. doi:10.1016/S0031-9422(74)80105-6.

Drewes SE, Mashimbye MJ. 1993. Flavanoids and triterpenoids from *Cassine papillosa* and the absolute configuration of 11, 11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxy-peltogynan. Phytochemistry. 32:1041–1044. doi:10.1016/0031-9422(93)85252-M.

Ferreira D, van der Merwe JP, Roux DG. 1974. Phytochemistry of the gum copal tree, *Trachylobium verrucosurn* (Gaertn.) Oliv. The first natural (-hydroxychalcone and 2,3-cis- and 2,3-trans-3-methoxyflavanones. J Chem Soc Perkin Trans. I:1492–1498. doi:10.1039/p19740001492.

Finney D. 1971. Probit analysis. 3 rd ed. New York: Cambridge University Press; p. 21–49.

Fukuyama Y, Hasegawa T, Toda M, Kodama M, Okazaki H. 1992. Structures of americanol A and Isoamericanol A having a neurotrophic property from the seeds of *Phytolacca americana*. Chem Pharm Bull. 40:252–254.

Haraguchi M, Motodome M, Gottlieb OR. 1988. Triterpenoid saponins and flavonol glycosides from *Phytolacca thyrsiflora*. Phytochemistry. 27:2291–2296. doi:10.1016/0031-9422(88)80145-6.

Hernández-Villegas MM, Borges-Argáez R, Rodríguez-Vivas RI, Torres-Acosta JFJ, Méndez-Gonzalez M, Cáceres-Farfan M. 2011. Ovicidal and larvicidal activity of the crude extracts from *Phytolacca icosaundra* and *Phytolacca rugosa*. Ciencia (Maracaibo). 22:53–66.

Koysomboon S, van Altena I, Kato S, Chantarapromma K. 2006. Antimycobacterial flavonoids from *Derris indica*. Phytochemistry. 67:1034–1040. doi:10.1016/j.phytochem.2006.03.019.
Lambert JDH, Wolde-Yohannas L, Maphubu L. 1985. Endod: potential for controlling schistosomiasis. BioScience. 35:364–366. doi:10.2307/1309905.

Lemma A. 1970. Laboratory and field evaluation of the molluscicidal properties of Phytolacca dodecandra. Bull World Health Organ. 42:597–612.

Lin Y-L, Shen C-C, Huang Y-J, Chang Y-Y. 2005. Homoflavonoids from Ophioglossum petiolatum. J Nat Prod. 68:381–384. doi:10.1021/np0401819.

Li X, Xu L, Wu P, Xie H, Huang Z, Ye W, Wei X. 2009. Prenylflavonols from the leaves of Macaranga sampsonii. Chem Pharm Bull. 57:495–498. doi:10.1248/cpb.57.495.

Malan E, Roux DG. 1979. Flavonoids from Distemonanthus benthamianus Baillon. Methoxylated flavones and inter-relationships of benthamianin, a [2]benzopyrano-[4,3-b][1]benzopyran. J Chem Soc Perkin Trans. I:2696–2703. doi:10.1039/p19790002696.

Malan JCS, Young DA, Steynberg JP, Ferreira D. 1990. Oligomeric flavanoids. Part 9. The first biflavanoids based on moplan and peltogynol as inceptive electrophiles. J Chem Soc Perkin Trans. I:219–225. doi:10.1039/p19900000219.

Mcpherson DD, Cordell GA, Soejarto DD, Pezzuto JM, Fong HHS. 1983. Peltogynoids and homoisoflavonoids from Caesalpinia pulcherrima. Phytochemistry. 22:2835–2838. doi:10.1016/S0031-9422(00)97708-2.

Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D, McLaughlin J. 1982. Brine shrimp: A convenient general bioassay for active plants constituents. Planta Med. 45:31–34. doi:10.1055/s-2007-971236.

Ravikiran G, Raju AB, Venugopal Y. 2011. Phytolacca americana: a review. Int J Res Pharm Biomed Sci. 2:942–946.

Razdan TK, Harkar S, Kachroo V, Koul GL. 1982. Phytolaccanol and epiacetylaleuritolic acid, two triterpenoids from Phytolacca acinosa. Phytochemistry. 21:2339–2342. doi:10.1016/0031-9422(82)85201-1.

Rzedowski J, Calderón de Rzedowski J. 2000. Notas sobre el género Phytolacca (Phytolaccaceae) en México. Acta Bot Mex. 53:49–66.

Santos-Lozano JA, Villagomez-Ibarra JR, Lopez-Ramirez A, Montiel-Jarillo G, Bautista-Avilica M, Gayoso-de Lucio JA, Velázquez-González C. 2010. Antisecretory activity of methanol and chloroform extracts from aerial parts and flowers of Phytolacca icosandra L. Rev CENIC Cienc Biol. 41:1–5.

Soliman HSM, Sobieh OA. 1999. Two spermicidal saponins and two flavonoids from berries of Phytolacca dioica L. Al-Azhar J Pharm Sci. 23:84–96.

Steinmann VW. 1997. Phytolacca icosandra L. (Phytolaccaceae): new to the continental United States. Madroño. 44:108–109.

Treyvaud V, Marston A, Dyatmiko W, Hostettmann K. 2000. Molluscicidal saponins from Phytolacca icosandra. Phytochemistry. 55:603–609. doi:10.1016/S0031-9422(00)00233-8.

Van Heerden FR, Brandt EV, Roux DG. 1979. Structure and synthesis of a derivative of fasciculiferin, a novel pelotergynoid from Acacia fasciculifera. Tetrahedron Lett. 20:4507–4510. doi:10.1016/S0040-4039(00)86634-X.

Waibel R, Benirschke G, Benirschke M, Achenbach H. 2003. Sesquineolignans and other constituents from the seeds of Joannesia princeps. Phytochemistry. 62:805–811. doi:10.1016/S0031-9422(02)00357-6.

Williams LAD, Rössner H, Conrad J, Möller W, Beifuss U, Chiba K, Nkurunziza JP, Kraus W. 2002. Selected secondary metabolites from the Phytolaccaceae and their biological/pharmaceutical significance. Recent Res Devel Phytochem. 6:13–68.

Woo WS. 1974. Steroids and pentacyclic triterpenoids from Phytolacca americana. Phytochemistry. 13:2887–2889. doi:10.1016/0031-9422(74)80271-2.

Woo WS, Kang SS, Seligmann O, Chari VM, Wagner H. 1980. The structure of new lignans from the seeds of Phytolacca americana. Tetrahedron Lett. 21:4255–4258. doi:10.1016/S0040-4039(00)92876-4.

Zou G-A, Su Z-H, Zhang H-W, Wang Y, Yang J-S, Zou Z-M. 2010. Flavonoids from the stems of Croton caudatus Geisel. var. tomentosus Hook. Molecules. 15:1097–1102. doi:10.3390/molecules15031097.