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A Molluscicidal Triterpenoid Saponin from the Fruits of Napoleonaea P. Beauv (Lecythidaceae)

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ABSTRACT: A new molluscicidal triterpenoid saponin, napoleonaside [**1**] has been isolated from the methanolic extract of the fruit of *Napoleonaea imperialis*. The structure of napoleonaside was established as 3β-O-[{β-D-glucopyranosyl(1→4)}{α-L-arabinopyranosyl(1→2)-β-D-glucopyranosyl(1→2)}-β-D-glucuronopyranosyl]-16α,22α,24,28-tetrahydroxyl-21-β-O-angeloxyolean-12-ene, by spectroscopic (IR, FABMS, ^{1}H and ^{13}C-NMR) methods. Napoleonaside was tested for its molluscicidal properties against *Biomphalaria glabrata* and was found to be one of the most potent naturally occurring plant molluscicides with activity of 0.4ppm (observed after 24hrs). © JASEM

Keywords: *Napoleonaea imperialis, Lecythidaceae,* triterpenoid saponin, napoleonaside, molluscicide.

Napoleonaea imperialis P. Beauv is one of the plants employed in ethnomedicine in Nigeria. The bark and the fruit pulp are used as a cough medicine and the raw bark is chewed for this effect. The seeds are toxic and the toxic property is attributed to unidentified glucose (Dalziel, 1937). Earlier work on the seeds reported the isolation of napoleogenol and napoleogenin (Kapundu et al., 1980). However, no report on the isolation and biological activity of saponins of *N. imperialis* has appeared. In this paper, the isolation and structural elucidation of Napoleonaside [**1**], a new molluscicidal saponin of *N. imperialis* is reported.

EXPERIMENTAL

General: TLC was carried out on Kieselgel 60 F_{254} precoated glass sheets (Merck) with $CHCl_3$-MeOH-H_2O (4:2:1). Detection was by spraying with 50% aqueous H_2SO_4 followed by heating for 2 min. For CC, silica gel 60 (70-230 mesh, Merck) was used. PC was carried out on Whatman No 1 paper (with n-BuOH - C_6H_6 – C_5H_5N - H_2O, 5:1:3:3) using the descending mode and aniline hydrogen phthalate as developer. Molluscicidal testing was achieved with *Biomphalaria glabrata* snails (according to Hostettmann, Kizu and Tomimori, 1982).

Spectral Data: The IR spectra were recorded on a Shimadzu IR-480 spectrophotometer in KBr pellets. NMR spectra were recorded at 75 MHz for ^{13}C, and 300 MHz, for ^{1}H in $CDCl_3$, chemical shifts (δ) are expressed in ppm with TMS as an internal standard, using Varian instrument VXR 300. FABMS was done on JMS-DX300.

Plant Material: Fruit of N. *imperialis* were collected between January and February, 1988 from University of Port Harcourt, botanical garden and voucher sample deposited at University of Port Harcourt herbarium.

Extraction and Isolation: Air-dried powdered fruits (1kg) of *N. imperialis* were defatted with petrol and extracted exhaustively with methanol saturated with n-BuOH. The methanol extract was concentrated in vacuo to give a residue (200g). A part (40g) of the residue was dissolved in MeOH (500ml), filtered and then added to ether (2000 ml) in drops to give a white precipitate. The ppt was filtered and dried to afford a brown powder (18g) which was positive to Liebermann-Burchard test for saponins. The crude saponin (5g) was chromatographed on a column of silica gel with n-BuOH – AcOH - H_2O (5:1:4) to give three fractions. After CC of third fraction (1g) on silica gel eluted with $CHCl_3$ – MeOH - H_2O (4:2:1), three compounds N1-01 (200mg), N1-02 (600mg) and N1-03 (140mg) were obtained. N1-02 (napoleonaside [**1**]) was homogenous on TLC (R_f 0.14). N1-01 and N1-03 were kept for later studies.

Napoleonaside [1]: White amorphous powder, mp 240-242^0 C ; ir (cm^{-1}): 3400-3200 (OH), 2900 (C-H), 1720 (C=O, from ester) 1700 (C=O from acid), 1690 (α, β-unsaturated C=O), 1610 (C=CH), 1380-1360,1260-1230 (C-H),1070-1020 (C-O or OH of alcohols): ^{1}H-nmr (δ): 0.78, 0.80, 1.20 (6H), 1.30, 1.40 (total 18H, 6x tert-CH_3), 1.95 (br, s, angeloyl α-CH_3) 2.15 (d, J=7, angeloyl β-CH_3), 4.85 to 5.00 (anomeric protons), 5.3 (m, 1H, C-12H), 5.85 (angeloyl, βH). ^{13}C-nmr (δ) : Triterpenoid moiety (C_1 to C_{30}) 38.88, 26.55, 91.05, 43.91, 56.44, 18.44, 33.32, 40.09, 46.83, 39.24, 24.16, 122.56, 143.10, 41.92, 34.95, 68.80, 47.90, 40.24, 47.90, 36.58, 81.50, 73.35, 22.80, 63.46, 15.91, 16.89, 27.67, 66.75, 29.95, 20.37, Angelic acid moiety 168.38,(CO), 129.21 (C),16.00 (C-$\underline{C}H_3$), 136.19

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(CH), 20.82 (CH-$\underline{C}H_3$). Glucuronopyranoic acid (C_1-C_6), 104.83, 79.63, 76.51, 81.49, 75.48, 171.82, Arabinose (1→2)105.88, 73.52, 74.79, 70.18, 63.02, Glucose(1→2) 104.53, 75.41, 78.01, 70.11, 78.52, 61.85 Glucose(1→4)104.98, 74.79, 78.01, 71.26, 77.70, 61.60. FABMS (m/z): 1242, 1241, 1218, 1142, 1109, 1079, 947, 877(a), 787, 364(b), 213.

*Acid Hydrolysis of Napoleonaside***:** A solution of [1] (60mg) in 2M HCl-dioxane (1:1, 10 cm^3) was heated under reflux for 6hr, then diluted with water and extracted with EtOAc. The organic layer was neutralized with $BaCO_3$ and the PC of concentrate (co-chromatographed with authentic samples) revealed the presence of β-D-glucose, L-arabinose and D-glucuronic acid.

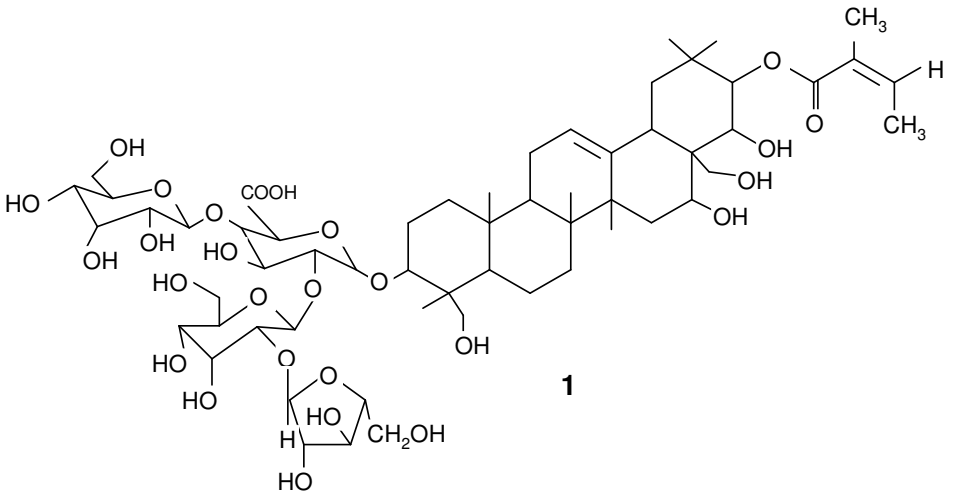
Basic Hydrolysis of Napoleonaside: A solution of [1] (120mg) in 1M KOH (10 cm^3) was heated under reflux for two hr. The reaction mixture was neutralized with 1M HCl and extracted with BuOH. After cc on silica gel using $CHCl_3$ – MeOH - H_2O (4:2:1), a polar compound was obtained whose ir and ^{13}C confirmed absence of angelic acid moiety. IR (KBr): 3400-3200 (OH), 1700 (-COOH), 1610 (trisubstituted double bond), 1159, 1030 cm^{-1}. ^{13}C-nmr (δ): :Triterpenoid moiety only (C_1-C_{30}) 38.15, 24.47, 90.00, 43.91, 55.44, 18.00, 32.59, 40.00 46.02, 36.58, 23.16, 122.56, 143.09, 40.93, 33.35, 66.50, 47.93, 39.65, 47.93, 34.96, 76.86, 76.34, 22.10, 63.43 ,15.90, 17.05, 27.67, 66.75, 29.95, 20.27.

RESULTS AND DISCUSSION

Column chromatography of the saponin mixture of the fruit of *Napoleonaea imperialis* gave Napoleonaside (**1**) and two other compounds not yet characterized. The three compounds were positive to Liebermann-Burchard tests for triterpenoidal saponins (Liebermann, 1985). Napoleonaside (1), m.pt 240-242^{0}C showed absorption bands (3400-3200, 1720, 1700, 1690 and 1610 cm^{-1}) due to hydroxyl, carboxylic, α, β-unsaturated ester groups as well as trisubstituted double bonds. The molecular formula, $C_{58}H_{90}O_{27}$, and MW of napoleonaside were concluded from the FABMS peaks at 1242 $[M+Na+H]^+$, 1241$[M+Na]^+$ and 1218 $[M^+]$. The peaks at m/z 1109 $[M+Na-132]^+$ and 1079 $[M+Na-162]^+$ correspond to the loss of terminal pentose and hexose units respectively. The peak at m/z 1142 $[M+Na-99]^+$ corresponds to the loss of one angelic acid moiety whereas RDA fragmentation of ring C characteristic of Δ^{12} pentacyclic triterpenoids (Djerassi et al.,1962), gave rise to peaks at m/z 877 (a) and 364 (b) .The H^1-nmr spectrum showed the presence of six quaternary methyl groups at δ 0.78 (3H), 0.80 (3H), 1.20(6H), 1.30 (3H), 1.40 (3H). One proton multiplet at δ 5.3 is characteristic of Δ^{12} proton in pentacyclic triterpene (Ojinnaka et al., 1984) and the signals at δ 1.95 (3H, α –CH_3), 2.15 (d, J=7, β-CH_3) and 5.85 (βH) reveal the presence of angeloyl group (Zhizhen et al., 1999). Anomeric signals were observed for the sugars between δ 4.80 and 5.00. Structure [**1**], is assigned to napoleonaside after analysis of its FABMS and the comparisons of the ^{13}C shielding data of napoleonaside and those of its oleanane analogue (Zhizhen et al., 1999). RDA fragmentation of napoleonaside gave 877 (a) and 364 (b). The peak at m/z 213 [b-2x17-18-99]$^+$ suggests that two secondary hydroxyls and one primary hydroxyl as well as one angelic acid moiety are attached to b fragment. The ^{13}C-nmr spectrum showed 58 carbon resonances. The presence of four monosaccharide moieties are indicated by four anomeric signals at δ 104.83 (glucuropyranoic acid), 104.53 and 104.98 (β-D-glucopyranose) (Zhizhen et al., 1999, Jing et. al., 2001) and 105.88 (L-arabinopyranose). The olefinic resonances at 143.10 and 122.56 corresponding to quaternary and methine carbon suggest the presence of Δ^{12} and confirm the oleanane skeleton. The presence of two primary and two secondary hydroxyls as well as one angeloxy and one glycoxy substituents in the aglycone moiety are deduced from the signals at δ 63.46, 66.75, 68.80, 73.35, 81.50 and 91.05 respectively. The chemical shifts for the hydroxyls in rings D and E are comparable to the reported ^{13}C shielding data for protoaescigenin (Zhizhen et al., 1999) and the secondary hydroxyls are assigned to 16α and 22α while the primary hydroxyls are assigned to 24 and 28. The existence of ester carbonyl at δ 168.38 (CO), a quaternary olefinic carbon at δ 136.19 (CH = **C**), an olefinic methine at 129.21 (**C**H=C) in addition to methyl signals at δ16.00 and 20.82 suggests the presence of angelic ester residue (Zhizhen et al., 1999). The ester residue (Singh et al., 1986) is assigned to C-21 because on alkaline hydrolysis the C-21 chemical shift is shielded (upfield) from δ 81.50 to δ 76.86. On acid hydrolysis napoleonaside, afforded β-D–glucose, β-D –glucuronic acid and L-arabinose (PC) in the ratio 2:1:1.

The glycosylation points were concluded from ^{13}C-nmr studies. The ^{13}C-nmr chemical shifts of methyl pyranosides, β-D- glucose (Seo et. al., 1978), L-arabinopyranoside (Kizu and Tomimori, 1982) and those of oleanolic acid (Doddrell et. al., 1974) as well as protoaescigenin (Zhizhen et al., 1999, Jing et al., 2001) are available. The glycosylation shifts of napoleonaside clearly indicated that glucuropyranoic acid was substituted at position 2 (79.63 ppm, downfield shift of ≈7.79 ppm) and at position 4 (81.49 ppm, downfield shift 8.79 ppm) with glucose

in comparison to the reported values for methyl-O-β-D-glucopyranoic acid (Gorin and Mazurek, 1975). The glucose at position 2 of glucuropyranoic acid is further glycosidated with L-arabinose at its position 2 (78.52 ppm, downfield shift of ≈ 4 ppm). The above conditions are further supported by the appearance of the upfield signals of the anomeric carbons of glucuropyranoic acid and the substituted glucose at δ 104.83 and 104.53 respectively. The ^{13}C-nmr spectrum gave evidence that napoleonaside, was glycosidated at C-3 (δ91.05). The C-3 for the unsubstituted aglycone (Seo et al., 1978; Kizu and Tomimori, 1982) usually appears at δ 80.3. From the spectroscopic properties, napoleonaside [**1],** is identified as 3β-O-[{β-D-glucopyranosyl (1→4)} {α-L-arabinopyranosyl (1→2)-β-D-glucopyranosyl(1→2)}β-D-glucuronopyranosyl]-16α, 22α, 24, 28-tetrahydroxyl-21-β-O-angeloxyolean-12-ene. Napoleonaside was tested for its molluscicidal properties (Hostettmann, Kizu and Tomimori, 1982) against *Biomphalaria glabrata*, the said vector of the alarming tropical disease, schistosomiasis and was found to be one of the most potent naturally occurring plant molluscicides (Hostettmann, 1992) with activity of 0.4 ppm (observed after 24hrs).



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