# 215. Further Saponins from *Phytolacca dodecandra* L'HERIT

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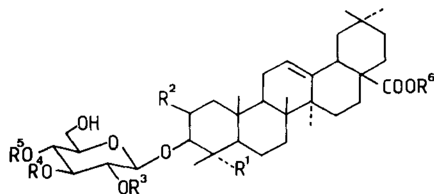
(15.IX.86)

Three new bidesmosidic saponins **1–3** have been isolated from the berries of *Phytolacca dodecandra* L'HERIT *(Phytolaccaceae)*. The structures have been established by spectroscopic ($^{1}$H-NMR, $^{13}$C-NMR, FAB-MS, DCI-MS, and GC/MS) and chemical methods (acid, basic, and enzymatic hydrolysis). Saponins **1** and **3** are tetra- and pentaglycosides of oleanolic acid, whereas **2** is a tetraglycoside of bayogenin. The corresponding prosapogenins **1a–3a**, obtained by cleavage of a glucose unit esterified at C(28), exhibited strong molluscicidal activity against schistosomiasis-transmitting snails.

**Introduction.** – Since the discovery of the molluscicidal properties of *Phytolacca dodecandra* L'HERIT by *Lemma* [1] in 1964, several phytochemical investigations of this plant have been undertaken. *Phytolacca dodecandra* is one of the most promising plants to be used for the local control of schistosomiasis. Water extracts of the berries have been evaluated in both laboratory and field trials [2]. Molluscicidal compounds have been isolated by *Parkhust* [3–5]. They are monodesmosidic saponins with oleanolic acid as




| Compound | R¹ | R² | R³ᵃ) | R⁴ᵇ) | R⁵ᵃ) | R⁶ᵃ) |
|---|---|---|---|---|---|---|
| **1** | $CH_3$ | H | H | Gal | Glc | Glc |
| **1a** | $CH_3$ | H | H | Gal | Glc | H |
| **1b** | $CH_3$ | H | H | H | Glc | H |
| **1c** | $CH_3$ | H | H | Gal | H | H |
| **2** | $CH_2OH$ | OH | H | Gal | Glc | Glc |
| **2a** | $CH_2OH$ | OH | H | Gal | Glc | H |
| **2b** | $CH_2OH$ | OH | H | H | Glc | H |
| **3** | $CH_3$ | H | Rha(1→2)Glc | H | Glc | Glc |
| **3a** | $CH_3$ | H | Rha(1→2)Glc | H | Glc | H |
| **3b** | $CH_3$ | H | Rha(1→2)Glc | H | H | H |
| **3c** | $CH_3$ | H | Glc | H | Glc | H |
| **3d** | $CH_3$ | H | Glc | H | H | H |

ᵃ) Rha = α-L-Rhamnopyranosyl; Glc = β-D-glucopyranosyl. ᵇ) Gal = β-D-Galactopyranosyl.

aglycone and various glycosidic chains. Recently, the proceedings of the 1983 workshop on *Phytolacca dodecandra* dealing with research on the chemical, toxicological, molluscicidal, and agronomic aspects of the plant have been published [6]. Meanwhile, we undertook the reinvestigation of *Phytolacca dodecandra* and isolated three new saponins from this species [7]. In the present paper, we report the structure elucidation of three further new bidesmosidic saponins.

**Results.** – The extraction and isolation of saponins **1–3** have already been described [7]. The MeOH extract of *Phytolacca dodecandra* was submitted to rotation locular counter-current chromatography (RLCC) [8] with the solvent system AcOEt/EtOH/$H_2O$ 4:2:4 in the ascending mode and afforded six fractions. Saponins **1–3** were isolated from *Fraction IV, V,* and *VI* and *VI* respectively, by reversed-phase chromatography on *RP-8*.

Acid hydrolysis of compounds **1** and **2** afforded the same sugars, *i.e.* galactose and glucose, whereas the aglycones were identified as oleanolic acid for **1** and bayogenin for **2** by comparison with authentic samples. Acid hydrolysis of **3** furnished oleanolic acid, glucose, and rhamnose. Basic hydrolysis of **1–3** yielded glucose and the monodesmosidic saponins **1a–3a**, respectively. The mol. wt. of the compounds and in part their structures were determined by MS.

The fast-atom-bombardment MS (FAB-MS [9]; thioglycerol matrix, negative-ion mode) of saponin **1** showed quasimolecular ions at *m/z* 1139 ($[M + Cl]^-$) and 1103 ($[M - H]^-$) indicating a mol. wt. of 1104. Additional signals at *m/z* 941 ($[(M - H) - 162]^-$), 779 ($[(M - H) - 324]^-$), 617 ($[(M - H) - 486]^-$), and 455 ($[(M - H) - 648]^-$) correspond to the successive loss of 4 hexosyl moieties. The desorption-chemical-ionization MS (DCI-MS [10]; reactant gas $NH_3$, positive-ion mode) of prosapogenin **1a** indicated a mol. wt. of 942, the quasimolecular ion ($[M + NH_4]^+$) appearing at *m/z* 960. Sugar-cleavage fragments are observed at *m/z* 798 ($[(M + NH_4) - 162]^+$), 636 ($[(M + NH_4) - 324]^+$), and 474 ($[(M + NH_4) - 486]^+$) corresponding to the successive loss of 3 hexosyl units.

Thus, **1** is a bidesmosidic saponin with 4 hexosyl units, 1 glucopyranosyl moiety being attached to the aglycone by an ester linkage. Saponin **1a** is the corresponding monodesmosidic oleanolic-acid triglycoside.

Saponin **2** was submitted to FAB-MS (negative-ion mode) and showed a similar fragmentation pattern to **1** with a shift of 32 *amu* resulting from the substitution of a $CH_3$ group at C(4) of the aglycone by $CH_2OH$ and an additional OH group attached at C(2). Thus, the following signals were observed: *m/z* 1135 (quasimolecular ion $[M - H]^-$), 973 ($[(M - H) - 162]^-$), 811 ($[(M - H) - 324]^-$), 649 ($[(M - H) - 486]^-$), and 487 ($[(M - H) - 648]^-$), the latter corresponding to the cleavage of sugar fragments. From the results of basic hydrolysis and MS, it appears that compound **2** is a bidesmosidic saponin with 4 hexosyl units, 1 glucose moiety being attached to the aglycone by an ester linkage. Saponin **2a** is the corresponding monodesmosidic bayogenin triglycoside. In the FAB-MS of **2a**, signals at *m/z* 973 ($[M - H]^-$), 811 ($[(M - H) - 162]^-$), 649 ($[(M - H) - 324]^-$), and 487 ($[(M - H) - 486]^-$) were observed. The FAB-MS of saponin **3** showed quasimolecular ions at *m/z* 1285 ($[M + Cl]^-$) and 1249 ($[M - H]^-$). A strong signal at *m/z* 1087 ($[(M - H) - 162]^-$) indicated the loss of one glucose unit confirming the result of basic hydrolysis. Additional signals could be observed at *m/z* 941 and 925 which correspond to the simultaneous cleavage of 1 rhamnosyl ($[(M - H) - 308]^-$) and 1 glucosyl ($[(M - H) - 324]^-$) moiety, respectively. These fragments indicated a branched-

sugar chain. Other peaks at $m/z$ 779 ($[(M - H) - 470]^-$), 617 $[(M - H) - 632]^-$), and 455 ($[(M - H) - 794]^-$) showed the successive loss of 3 glucopyranosyl units. The same sugar-cleavage fragments could be observed in the FAB-MS of the partially hydrolyzed saponin **3a** with 1 hexosyl unit less. Confirmation of the sugar sequence of **3** was obtained by DCI-MS (reactant gas $NH_3$, positive-ion mode). A quasimolecular ion appeared at $m/z$ 1268 ($[M + NH_4]^+$). The signal at $m/z$ 1106 ($[(M + NH_4) - 162]^+$) corresponds to the loss of the glucopyranosyl unit attached at C(28) of the aglycone. The signals at $m/z$ 960 ($[(M + NH_4) - 308]^+$) and 944 ($[(M + NH_4) - 324]^+$) confirmed the simultaneous cleavage of 1 rhamnosyl and 1 glucosyl moiety. Signals for sugar fragments at $m/z$ 326 ($[308 + NH_4]^+$) and 342 ($[324 + NH_4]^+$) verified these eliminations. Thus, compound **3** is a pentaglycosyl bidesmosidic saponin with a glucose unit attached at C(28) of the aglycone. The partially hydrolyzed compound **3a** is the corresponding prosapogenin with a branched-sugar chain formed of 3 glucose and 1 rhamnose units, terminal sugars being rhamnose and glucose.

The positions of attachment of the sugar chains to the aglycones of **1**–**3** were established by $^{13}$C-NMR spectroscopy and the $\beta$-D-pyranosyl and $\alpha$-L-pyranosyl configurations of the glucose, galactose, and rhamnose moieties were determined by $^1$H- and $^{13}$C-NMR. In all the isolated saponins, a sugar chain was linked to the aglycone at C(3): the C(3) signals in compounds **1** and **3** were observed at 89.4 ppm, whereas the chemical shift of this C-atom was 78.7 ppm in oleanolic acid [11]; C(3) of **2** appeared at 83.1 ppm, whereas in bayogenin, this C-atom could be observed at 73.5 ppm [12]. The free COOH groups appeared between 180.1–180.4 ppm in monodesmosidic saponins **1a**–**3a**, whereas when esterified with a glucosyl moiety, C(28) signals could be observed between 176.3–176.6 ppm (bidesmosidic saponins **1**–**3**).

The interglycosidic linkages were established by combination of $^{13}$C-NMR spectroscopy (chemical shifts, see *Table*), GC/MS analysis of the partially methylated alditol acetates (obtained by permethylation and hydrolysis of the saponins, followed by reduction and acetylation of the sugars), and partial acid (saponin **1**–**3**) and enzymatic hydrolysis (saponin **3**).

GC/MS of the partially methylated alditol acetates from saponin **1a** (see *Exper. Part*) indicated a terminal glucose, a terminal galactose, and a 3,4-*O*-disubstituted glucose moiety for **1a**. In the MS of the terminal-sugar derivatives, all the signals could be attributed to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol by comparison with fragmentation schemes of the literature [13]. The glucose and galactose derivatives were differentiated by their respective retention times in GC. The presence of a signal at $m/z$ 117 (77%) which does not exist for a 2,4-*O*-disubstituted glucose moiety indicates that the terminal sugars are linked to the inner glucosyl moiety at positions 3 and 4 [13]. All the signals for a 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylhexitol derived from the inner sugar could be identified.

From GC/MS analysis, it was, however, not possible to determine which sugar is attached at which position in **1a**. In order to resolve this question, saponin **1a** was submitted to partial mild hydrolysis (see *Exper. Part*). A mixture of two diglycosides was obtained which was difficult to separate. Finally, pure **1b** and **1c** were obtained by semi-preparative HPLC on a *RP-8* column with $MeCN/H_2O$ 38:62 as eluent. The FAB-MS of **1b** and **1c** showed molecular ions at $m/z$ 779 ($[M - H]^-$) indicating a mol. wt. for both compounds of 780. Signals at $m/z$ 617 ($[(M - H) - 162]^-$) and 455

Table. *$^{13}$C-NMR Chemical Shifts of Sugar Moieties in ($D_5$)Pyridine*

| | | **1** | **1a** | **1b** | **2** | **2a** | **2b** | **3** | **3a** | **3b** | **3c** | **3d** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-Glc[a]) | C(1) | 104.8 | 104.9 | 105.0 | 104.7[d]) | 104.7[d]) | 105.0 | 104.6[d]) | 104.7[d]) | 105.1 | 104.9 | 104.9 |
| | C(2) | 73.4 | 73.2 | 74.9[d]) | 72.8 | 72.9 | 74.8[d]) | 81.6 | 81.6 | 79.4 | 81.5 | 83.5 |
| | C(3) | 82.9 | 82.9 | 76.4 | 82.8 | 82.9 | 76.8 | 74.9 | 74.9 | 77.8 | 74.9 | 77.7 |
| | C(4) | 77.4 | 77.4 | 81.6 | 77.5 | 77.6 | 81.1 | 79.4 | 79.4 | 73.1 | 81.2 | 71.9[d]) |
| | C(5) | 77.2 | 77.2 | 76.9 | 77.1 | 77.2 | 76.4 | 78.1 | 77.5 | 79.4 | 77.1 | 78.0[e]) |
| | C(6) | 62.0[d]) | 62.0[d]) | 64.7 | 62.2[e]) | 62.0[e]) | 62.0[e]) | 63.2 | 63.5 | 63.6 | 62.8[d]) | 62.9[f]) |
| Glc[a]) | C(1) | 105.7 | 105.7 | 106.6 | 104.8[d]) | 104.9[d]) | 105.5 | 104.7[d]) | 104.8[d]) | | 105.3 | 105.9 |
| terminal | C(2) | 75.0 | 75.0 | 75.2[d]) | 75.0 | 75.1 | 75.0[d]) | 76.0 | 76.0 | | 76.6 | 76.8 |
| | C(3) | 78.1 | 78.2 | 78.5 | 78.2 | 78.2 | 78.5 | 78.3 | 78.3 | | 78.2 | 78.4 |
| | C(4) | 71.0 | 70.9 | 71.5 | 70.9 | 70.9 | 71.5 | 71.4 | 71.4 | | 71.7 | 72.1[d]) |
| | C(5) | 77.9 | 77.9 | 78.2 | 77.8 | 77.9 | 78.2 | 78.5 | 78.4 | | 78.4 | 77.9[e]) |
| | C(6) | 62.4[d]) | 62.1[d]) | 62.4 | 62.4[e]) | 62.3[e]) | 62.4[e]) | 62.2 | 62.2 | | 62.2[d]) | 63.0[f]) |
| Gal[b]) | C(1) | 102.5 | 102.5 | | 102.6 | 102.6 | | | | | | |
| terminal | C(2) | 72.7 | 72.8 | | 72.6 | 72.7 | | | | | | |
| | C(3) | 74.6 | 74.5 | | 74.4 | 74.5 | | | | | | |
| | C(4) | 70.0 | 69.9 | | 69.9 | 70.0 | | | | | | |
| | C(5) | 75.7 | 75.7 | | 75.3 | 75.3 | | | | | | |
| | C(6) | 61.8 | 61.8 | | 61.8 | 61.4 | | | | | | |
| Glc[a]) | C(1) | | | | | | | 101.7 | 101.7 | 102.9 | 105.0[g]) | |
| inner | C(2) | | | | | | | 79.1 | 78.5 | 79.7 | 76.1 | |
| | C(3) | | | | | | | 77.1 | 77.4 | 77.3 | 77.8 | |
| | C(4) | | | | | | | 72.6 | 72.9 | 72.7 | 71.8 | |
| | C(5) | | | | | | | 78.2 | 78.1 | 79.0 | 78.3 | |
| | C(6) | | | | | | | 62.3 | 62.1 | 63.0 | 62.1 | |
| Rha[c]) | C(1) | | | | | | | 101.7 | 101.8 | 102.0 | | |
| terminal | C(2) | | | | | | | 72.6 | 72.6 | 72.4 | | |
| | C(3) | | | | | | | 72.5 | 72.5 | 72.1 | | |
| | C(4) | | | | | | | 74.1 | 74.3 | 74.4 | | |
| | C(5) | | | | | | | 69.4 | 69.4 | 69.6 | | |
| | C(6) | | | | | | | 19.0 | 18.6 | 18.6 | | |
| 28-Glc[a]) | C(1) | 95.7 | | | 95.7 | | | 95.7 | | | | |
| | C(2) | 74.1 | | | 74.1 | | | 74.3 | | | | |
| | C(3) | 78.9 | | | 78.1 | | | 78.2 | | | | |
| | C(4) | 71.4 | | | 70.5 | | | 71.3 | | | | |
| | C(5) | 79.1 | | | 79.1 | | | 78.8 | | | | |
| | C(6) | 62.4 | | | 61.9 | | | 62.4 | | | | |

[a]) Glc = β-D-Glucopyranosyl.
[b]) Gal = β-D-Galactopyranosyl.
[c]) Rha = α-L-Rhamnopyranosyl.
[d])[e])[f]) Signals in the verticals columns may be interchanged.
[g]) Data of Glc at 2-*O* of the 3-Glc moiety.

($[(M - H) - 324]^-$) corresponded to the subsequent loss of 2 hexosyl moieties. Acid hydrolysis of **1b** furnished glucose and oleanolic acid and of **1c** glucose, galactose, and oleanolic acid. The interglycosidic linkage in compound **1b** was determined by GC/MS of the partially methylated alditol acetates and by $^{13}$C-NMR (see *Table*). The chemical shifts of the C-atoms of the inner β-D-glucopyranosyl moiety of **1b** clearly indicated that the terminal glucosyl unit is attached at position 4 (downfield shift for C(4) by 10.1 to 81.6

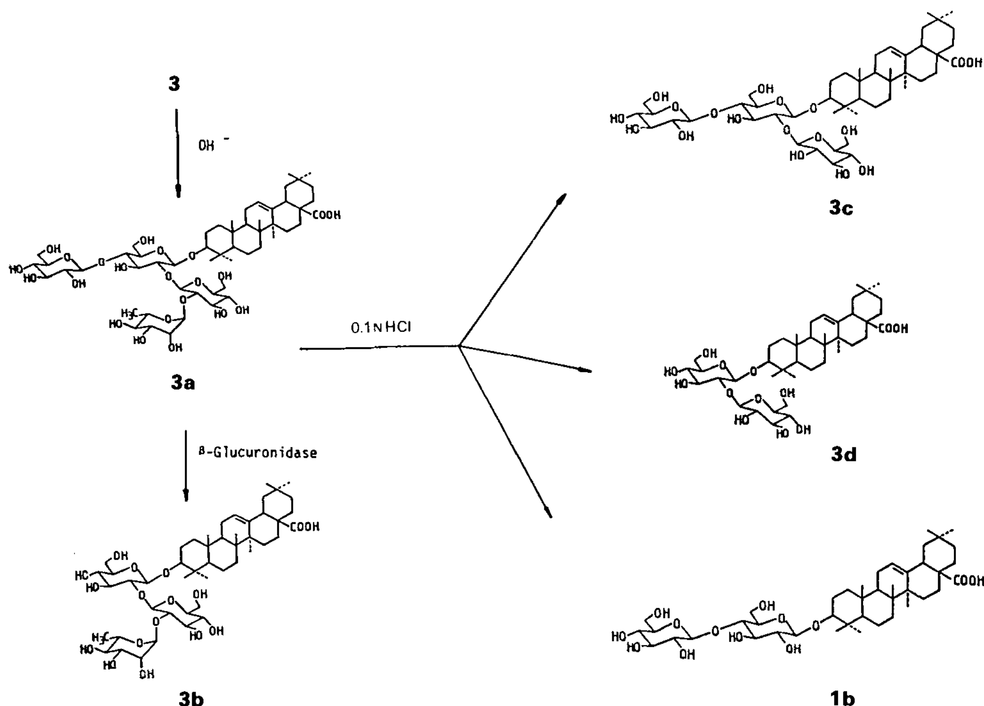
ppm; upfield shift for C(3) an C(5) by 2.1 and 1.3 ppm, resp.; other C-atoms almost unaffected). These results are in agreement with the glycosylation rule previously established by *Konishi et al.* [14]. Thus, the structure of **1b** is established as *3-O-[O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]oleanolic acid,* and consequently **1c** is *3-O-[O-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl]oleanolic acid.* Structure **1a** is, therefore, deduced to be *3-O-{O-β-D-galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolic acid.* Compound **1** is the corresponding bidesmosidic saponin with one glucosyl unit linked to the aglycone at C(28).

The $^{13}$C-NMR signals arising from the sugar moieties of saponin **2a** have shifts very similar to those of the sugar moieties of **1a**, indicating the same branched-sugar chain. This was confirmed by GC/MS analysis of the methylated alditol acetates obtained from **2a** revealing terminal galactopyranose, terminal glucopyranose, and 3,4-*O*-disubstituted glucopyranose moieties for **2a**. The same sugar derivatives 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol and 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylhexitol were identified for **1a**. However, it remained to determine where the glucose and the galactose moieties are attached. Therefore, mild acid partial hydrolysis of **2a** was realized, and **2b** was obtained as the major compound. The hydrolyzed products were separated from the original saponin by low-pressure liquid chromatography on a *RP-8* column with MeOH/$H_2O$ 6:4 as eluent. Compound **2b** was identical (co-TLC, HPLC, $^{13}$C-NMR) to *3-O-[O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]bayogenin,* isolated previously from *Phytolacca dodecandra* by *Domon* and *Hostettmann* [7]. Consequently, the structure of **2a** is established as *3-O-{O-β-D-galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}bayogenin.* Saponin **2** is the corresponding bidesmosidic saponin with one glucopyranosyl unit linked to bayogenin.

For saponin **3**, the interglycosidic linkage was determined as above in combination with results of enzymatic and partial acid hydrolysis (see *Scheme*). Enzymatic hydrolysis of **3a** with β-glucuronidase (conditions, see *Exper. Part*) afforded glucose and saponin **3b**. The FAB-MS of **3b** showed quasimolecular ions at *m/z* 961 ($[M + Cl]^-$) and 925 ($[M - H]^-$) indicating a mol. wt. of 926. Additional signals at *m/z* 779 ($[(M - H) - 146]$), 617 ($[(M - H) - 308]^-$), and 455 ($[(M - H) - 470]^-$) corresponded to the cleavage of 1 rhamnosyl and 1 and 2 hexosyl moieties, respectively. Thus, the sugar chain is linear with a rhamnose as terminal unit. Enzymatic hydrolysis of **3a** permitted the elimination of 1 terminal glucose. The interglycosidic linkage of **3b** was established on the basis of $^{13}$C-NMR and GC/MS of the alditol-acetate mixture obtained from the partially methylated saponin derived from **3b**. By GC/MS of this mixture, 2 GC peaks could be identified; one was attributable to a derivative of the terminal rhamnopyranose, *i.e.* to 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylhexitol, and the other to a 2-*O*-substituted glucopyranose derivative, *i.e.* to 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylhexitol. In the $^{13}$C-NMR spectra of **3b**, chemical shifts of the C-atoms of the 2 inner β-glucopyranosyl moieties indicated their substitution at position 2 (the C(2) signal is shifted downfield to 79.7 ppm). Thus, **3b** is identical to *3-O-[O-α-L-rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic acid.* This saponin was previously described in *Phytolacca dodecandra* as lemmatoxin C by *Parkhust et al.* [4].

To complete the structure of saponin **3a**, partial mild acid hydrolysis was necessary in order to determine the position of linkage of the last unit of glucose which was cleaved by β-glucuronidase to give **3b**. Under mild acid conditions, saponin **3a** furnished **3c**, **3d**, and

Scheme. *Structure Determination of* **3**




**1b**. These compounds were separated by reversed-phase (*RP-8*) chromatography. A low-pressure system was used with $MeOH/H_2O$ 65:35 as eluent. The mol. wt. of **3c** was determined by DCI-MS. The MS of **3c** (reactant gas $NH_3$, positive-ion mode) showed a quasimolecular ion at $m/z$ 960 ($[M + NH_4]^+$). Addition signals were observed at $m/z$ 798 ($[(M + NH_4) - 162]^+$) and 636 ($[(M + NH_4) - 324]^+$). Complementary fragments for 3 and 2 glucosyl units appeared at $m/z$ 504 ($[486 + NH_4]^+$) and 342 ($[324 + NH_4]^+$) respectively. $^{13}$C-NMR signals arising from the sugar moieties of saponin **3c** had shifts very similar to those of oleanoglycotoxin A, previously described in [7]. Thus, the structure of **3c** is established as *3-O-[2',4'-di-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl]oleanolic acid*. The other products **3d** and **1b** obtained from partial hydrolysis of **3a** confirmed the structure of **3c**. Saponin **3d** was identified as *3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic acid*. In the $^{13}$C-NMR spectrum of **3d**, the C(2) signal of the inner β-D-glucopyranosyl moiety was shifted downfield by 6.7 to 83.5 ppm. The anomeric C-atom and the C(3) signal were shifted upfield by 1 and 0.7 ppm, respectively. The MS fragmentation signals in the GC/MS of the alditol acetates obtained from **3d** could be attributed to the derivatives of terminal glucopyranose and 2-*O*-substituted glucopyranose. By combination of the results of enzymatic and mild acid hydrolysis, **3a** was identified as *3-O-{O-α-L-rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolic acid*. Compound **3** is the corresponding bidesmosidic saponin with an additional glucopyranosyl unit linked to the aglycone at C(28).

**Discussion.** – Pursuing our phytochemical reinvestigation of *Phytolacca dodecandra* berries, the unreported bidesmosidic saponins **1–3** have been isolated from the MeOH extract. Saponin **1a** obtained after basic hydrolysis of **1** has already been described as lemmatoxin by *Parkhust et al.* [5]. Saponin **3b** named lemmatoxin C by the same authors was produced by enzymatic hydrolysis of **3a**. Saponin **2b** has been isolated from *Phytolacca dodecandra* by *Domon* and *Hostettmann* [7]. Among the saponins obtained by chemical degradation, **1b**, **1c**, **2a**, **3a**, and **3d** have been reported for the first time. The results on the molluscicidal activity of the bidesmosidic saponins **1–3** showed that they were not toxic to *Biomphalaria glabrata* snails [15], the intermediate host in schistosomiasis. Only the monodesmosidic saponins were molluscicidal. Lemmatoxin (**1a**) was the most active compound and killed snails at a concentration of 3 ppm within 24 h. Saponins **1b**, **2b**, **3b**, **3c**, and **3d** were active at concentrations of 10, 12, 25, 6, and 6 ppm, respectively. The other products were not molluscicidal at 25 ppm. The spermicidal properties of the saponins were also tested [16]. Lemmatoxin (**1a**), oleanoglycotoxin A (**3c**), and saponin **2a** were active against human spermatozoids at concentrations of 0.05, 0.3, and 0.1 mg/ml, respectively. With these results, we have completed the structure determination of the main saponins of *Phytolacca dodecandra*. Preliminary work showed that saponins of a crude extract of *Phytolacca dodecandra* could be analysed by HPLC on a *RP-8* column with $MeCN/H_2O$ [17]. We are now investigating different strains of cultivated *Phytolacca dodecandra* by this method in an effort to quantify the content of active saponins. Furthermore, toxicological tests are underway to determine whether large-scale application of *Phytolacca dodecandra* for the control of schistosomiasis is feasible.

Financial support has been provided by the *UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases* and the *Swiss National Science Foundation*. Prof. *Teklemariam Aeyle*, Addis Ababa, is gratefully acknowledged for providing plant material. Thanks are also due to Prof. *J. Lauterwein* ($^1$H- and $^{13}$C-NMR), Lausanne, to Prof. *W.J. Richter* (FAB-MS), *Ciba-Geigy AG*, Basel, to Mr. *D. Chollet* (DCI-MS), *Zyma SA*, Nyon, to *Spectrospin AG*, Fällanden, and to *Varian AG*, Zug, for spectral measurements.

## Experimental Part

*General.* Column chromatography: *Lobar Lichroprep RP-8* column (40–63 µm; i.d. 2.5 × 27 cm; *Merck*, Darmstadt) equipped with a *Duramatic-80* pump (*Chemie und Filter*, Regensdorf) and *Sephadex LH 20* column (i.d. 2.5 × 30 cm) (*Pharmacia Fine Chemicals*). Anal. HPLC: *Spectra-Physics-8700* pump, *Rheodyne* injector, and *LKB-2151* UV detector; 7-µm *RP-8* column (i.d. 4.6 mm × 25 cm; *Knauer*). Semi-prep. HPLC: *Waters 6000A* pump, *U6K* injector, and *Pye-Unicam-LC* UV detector; 7-µm *RP-8* colum (i.d. 16 mm × 25 cm; *Knauer*). Final purification of saponins was achieved on a 2.5 × 45 cm *Sephadex LH 20* column. GC/MS: *Dani-6500* apparatus coupled with a *Nermag R 3010* spectrometer using a 0.22 mm × 25 m fused silica column packed with *SE 54* (injection temp. 210°, column temp. 130°/3 min up to 250°) or a 0.023 mm × 25 m column packed with *OV-225* (injection temp. 220°, column temp. 150°/1 min and 5°/min up to 210°). TLC: silica-gel-precoated Al sheets (*Merck*, Darmstadt) with $CHCl_3/MeOH/H_2O$ 58:35:7 (system 1) or 70:30:3 (system 2) and *RP-8*-precoated glass plates (HPTLC, *Merck*, Darmstadt) with $MeOH/H_2O$ 60–40; detection with *Godin* reagent [18] (bayogenin derivatives show a blue colour, whereas those of oleanolic acid are purple). M.p.: *Mettler FP 80/82* hot-stage apparatus; uncorrected. $^1$H- and $^{13}$C-NMR: in ($D_5$)pyridine; *Bruker WP-200* instrument at 200 and 50.29 MHz, resp.; for $^{13}$C-NMR, *Bruker WP-360*; *Varian-XL-400* apparatus at 90.5 and 101 MHz, resp.; TMS was used as internal standard. Desorption-chemical-ionization (DCI) MS: *Ribermag-R10-10B* quadrupole instrument with $NH_3$ as reactant gas. Fast-atom bombardment (FAB)-MS: negative-ion mode, *ZAB S1* spectrometer; samples were suspended in thioglycerol, and the target was bombarded with 5-keV Xe-atoms.

*Extraction and Isolation.* Dried berries of *Phytolacca dodecandra* (70 g) collected in Ethiopia (strain 17) were extracted as described in [7]. The MeOH extract (22 g) was separated and fractionated under the conditions described previously. *Fraction IV* (1225 mg) gave 195 mg of saponin **1**, purification of *Fractions V* and *VI* afforded saponin **2** (165 mg), and chromatography of *Fraction VI* gave 470 mg of saponin **3**.

*Acid Hydrolysis.* The saponin (2 mg) was refluxed in 4N HCl (10 ml) for 4 h. The mixture was extracted with AcOEt. The org. layer was checked by TLC for the aglycones with $(i\text{-}Pr)_2O$/acetone 70:30. The aq. layer was adjusted to pH 6 with $NaHCO_3$. After evaporation to dryness, the sugars were extracted with pyridine from the residue and analysed by TLC on silica gel with AcOEt/MeOH/$H_2O$/AcOH 65:15:15:20, detection with *p*-anisidine phthalate.

*Basic Hydrolysis.* As in [7].

*Partial Acid Hydrolysis.* The saponin (150 mg of **1a**, 120 mg of **2a**, or 150 mg of **3a**) was refluxed in 0.1N HCl (50 ml) for 90, 30, and 45 min, resp. The mixture was extracted with $H_2O$-sat. BuOH (2 × 50 ml). The org. phase was washed with $H_2O$ and evaporated. The partially hydrolysed saponin was purified on a *Sephadex LH 20* column, eluted with MeOH, and then treated as indicated previously.

*Enzymatic Hydrolysis.* Saponin **3a** (175 mg) and β-D-glucuronidase (50 mg) from *Helix pomatia* (*G-1512; Sigma Chemical*, St. Louis) were dissolved in acetate buffer (100 ml) of pH 5.5. The mixture was kept at 37° for 2 days, extracted with $H_2O$-sat. BuOH (2 × 100 ml), washed with $H_2O$, and evaporated. Purification was made on a *Sephadex LH 20* column (3 × 50 cm) with MeOH as eluent: 120 mg of pure **3b**.

*Permethylation.* The saponin (5 mg) was dissolved in dry DMSO (0.4 ml) and stirred at r.t. with dry *t*-BuONa (50 mg), dry, finely powdered NaOH (10 mg), and $CH_3I$ (0.3 ml) for 90 min. The mixture was then extracted with $Et_2O$ (15 ml), washed with a sat. NaCl soln., and evaporated. In order to obtain free sugars, the saponin was refluxed in 2N HCl/dioxane 1:1 for 6 h. The soln. was adjusted to pH 6 wih $Na_2CO_3$ while it was kept in an ice bath. The mixture was filtered and extracted with AcOEt (2 × 5 ml) and pyridine (2 × 5 ml). The combined extracts were evaporated for reduction and acetylation.

*Reduction and Acetylation.* The permethylated product was treated with 0.8 ml of a 20% aq. soln. of $NaBD_4$. The mixture was stirred at r.t. for 90 min and then acidified with AcOH, evaporated, and subjected to azeotropic distillation with toluene (3 × 5 ml). The resulting methylated alditol mixture was dissolved in $Ac_2O$/pyridine 1:1 and stirred at r.t. for 36 h. The soln. was evaporated and the methylated alditol acetates were analysed by GC and GC/MS.

*β-D-Glucopyranosyl 3-O-{O-β-D-Galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolate* (= *β-D-Glucopyranosyl 3β-{[O-β-D-Galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]oxy}olean-12-en-28-oate;* **1**). Amorphous, white powder, m.p. 235–240° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.19. $^{13}$C-NMR (50.3 MHz, ($D_5$)pyridine): δ's of the aglycone correspond to those previously described for oleanolic acid [11]; signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 1139 ($[M + Cl]^-$), 1103 ($[M - H]^-$), 941 ($[(M - H) - 162]^-$), 779 ($[(M - H) - 324]^-$), 617 ($[(M - H) - 486]^-$), 455 ($[(M - H) - 648]^-$).

Acid hydrolysis of **1** afforded D-glucose, D-galactose, and oleanolic acid.

*3-O-{O-β-D-Galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolic Acid* (= *3β-{[O-β-D-Galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]oxy}olean-12-en-28-oic Acid;* **1a**) was obtained after basic hydrolysis of **1**. Amorphous white powder, m.p. 250–254° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.43. $^{13}$C-NMR (90.5 MHz, ($D_5$)pyridine): δ's of the aglycone identical to those of **1** except for the signal at C(28) which appears at 180.1 ppm; signals of the sugar moieties, see *Table*. DCI-MS ($NH_3$, positive ions): 960 ($[M + NH_4]^+$), 798 ($[(M + NH_4) - 162]^+$), 636 ($[(M + NH_4) - 324]^+$), 474 ($[(M + NH_4) - 486]^+$).

*3-O-[O-β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl]oleanolic Acid* (= *3β-[(O-β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl)oxy]olean-12-en-28-oic Acid;* **1b**) was obtained by partial acid hydrolysis of **1a**. Amorphous, white powder, m.p. 242–246° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.75. $^{13}$C-NMR (101 MHz, ($D_5$)pyridine): δ's of the aglycone identical to those of **1a**; signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 779 ($[M - H]^-$), 617 ($[(M - H) - 162]^-$), 455 ($[(M - H) - 324]^-$).

*28-O-(β-D-Glucopyranosyl)-3-O-{O-β-D-galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}bayogenin* (= *β-D-Glycopyranosyl 3β-{[O-β-D-Galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]oxy}-2β,23-dihydroxyolean-12-en-28-oate;* **2**). Amorphous, white powder, m.p. 323–327° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.1. $^{13}$C-NMR (90.5 MHz, ($D_5$)pyridine): δ's of the aglycone correspond to those described for bayogenin [7]; signals for the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 1135 ($[M - H]^-$), 973 ($[(M - H) - 162]^-$), 811 ($[(M - H) - 324]^-$), 649 ($[(M - H) - 486]^-$), 487 ($[(M - H) - 648]^-$).

Acid hydrolysis of **2** afforded D-glucose, D-galactose, and bayogenin.

*3-O-[O-β-D-Galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]bayogenin* ( = *3β-{[O-β-D-Galactopyranosyl-(1→3)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]oxy}-2β,23-dihydroxy-olean-12-en-28-oic Acid;* **2a**) was obtained from basic hydrolysis of **2**. Amorphous, white powder, 250–255° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.29. $^{13}$C-NMR (90.5 MHz, ($D_5$)pyridine): chemical shifts of the aglycone are identical with those of **2** except for the signal of C(28) which appears at 180.3 ppm; sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 973 ([$M$ – H]$^-$), 811 ([($M$ – H) – 162]$^-$), 649 ([($M$ – H) – 324]$^-$), 487 ([($M$ – H) – 486]$^-$).

*3-O-[O-β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl]bayogenin* ( = *3β-[(O-β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl)oxy]-2β,23-dihydroxyolean-12-en-28-oic Acid;* **2b**) was obtained from partial acid hydrolysis of **2a**. Amorphous, white powder, m.p. 250–260° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.62. $^{13}$C-NMR (101 MHz, ($D_5$)pyridine): chemical shifts of **2b** were identical to those of **1a** described in [7].

*β-D-Glucopyranosyl 3-O-{O-α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2))-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolate* ( = *β-D-Glucopyranosyl 3β-{[O-α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]oxy}olean-12-en-28-oate;* **3**). Amorphous, white powder, m.p. 254–258°. TLC ($SiO_2$, system 2): $R_f$ 0.06. $^{13}$C-NMR (50.3 MHz, ($D_5$)pyridine): chemical shifts of the aglycone were identical to those of **1**; signals for the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 1285 ([$M$ + Cl]$^-$), 1249 ([$M$ – H]$^-$), 1087 ([($M$ – H) – 162]$^-$), 941 ([($M$ – H) – 308]$^-$), 925 ([($M$ – H) – 324]$^-$), 779 ([($M$ – H) – 470]$^-$), 617 ([($M$ – H) – 632]$^-$). DCI-MS ($NH_3$, positive ions): 1268 ([$M$ + $NH_4$]$^+$), 1106 ([($M$ + $NH_4$) – 162]$^+$), 960 ([($M$ + $NH_4$) – 308]$^+$), 944 ([($M$ + $NH_4$) – 324]$^+$), 342 ([324 + $NH_4$]$^+$), 326 ([308 + $NH_4$]$^+$). Acid hydrolysis of **3** afforded oleanolic acid, D-glucose, and L-rhamnose.

*3-O-{O-α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolic Acid* ( = *3β-{[O-α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl]oxy}olean-12-en-28-oic Acid* (**3a**) was obtained after basic hydrolysis of **3**. Amorphous, white powder, m.p. 228–234° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.14. $^{13}$C-NMR (90.5 MHz, ($D_5$)pyridine): chemical shifts of the aglycone correspond to those of **1a**; signals for the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 1087 ([$M$ – H]$^-$), 941 ([($M$ – H) – 146]$^-$), 925 ([($M$ – H) – 162]$^-$), 779 ([($M$ – H) – 308]$^-$), 617 ([($M$ – H) – 632]$^-$), 455 ([($M$ – H) – 794]$^-$).

*3-O-[O-α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic Acid* ( = *3β-[(O-α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]olean-12-en-28-oic Acid;* **3b**) was obtained by enzymatic hydrolysis of **3a**. Amorphous, white powder, m.p. 227–234° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.42. $^{13}$C-NMR (50.3 MHz, ($D_5$)pyridine): chemical shifts of the aglycone were identical to those of **3a**; sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 961 ([$M$ + Cl]$^-$), 925 ([$M$ – H]$^-$), 779 ([($M$ – H) – 146]$^-$), 617 ([($M$ – H) – 308]$^-$), 455 ([($M$ – H) – 470]$^-$).

*3-O-[2',4'-Di-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl]oleanolic Acid* ( = *3β-[(2',4'-Di-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)oxy]olean-12-en-28-oic Acid;* **3c**): identical to **2a** described in [7].

*3-O-[O-β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic Acid* ( = *3β-[(O-β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]olean-12-en-28-oic Acid;* **3d**) was obtained from partial acid hydrolysis of **3a**. Amorphous, white powder, m.p. 240–245° (dec.). TLC ($SiO_2$, system 2): $R_f$ 0.74. $^{13}$C-NMR (50.3 MHz, ($D_5$)pyridine): chemical shifts of the aglycone correspond to those of **3a**; signals of the sugar moieties, see *Table*. DCI-MS ($NH_3$, positive ions): 798 ([$M$ + $NH_4$]$^+$), 636 ([($M$ + $NH_4$) – 162]$^+$), 474 ([($M$ + $NH_4$) – 324]$^+$).

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