

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

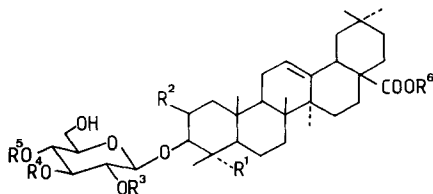
by Anne-Christine Dorsaz and Kurt Hostettmann*

Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie de l'Université de Lausanne, 2, rue Vuillermet,
CH-1005 Lausanne

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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 ($^1\text{H-NMR}$, $^{13}\text{C-NMR}$, 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 Parkhurst [3–5]. They are monodesmosidic saponins with oleanolic acid as



Compound	R ¹	R ²	R ^{3a)}	R ^{4b)}	R ^{5a)}	R ^{6a)}
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 ₂ OH	OH	H	Gal	Glc	Glc
2a	CH ₂ OH	OH	H	Gal	Glc	H
2b	CH ₂ OH	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/H₂O 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 NH₃, 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 CH₃ group at C(4) of the aglycone by CH₂OH 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 ($[M + \text{NH}_4]^+$). The signal at m/z 1106 ($[(M + \text{NH}_4) - 162]^+$) corresponds to the loss of the glucopyranosyl unit attached at C(28) of the aglycone. The signals at m/z 960 ($[(M + \text{NH}_4) - 308]^+$) and 944 ($[(M + \text{NH}_4) - 324]^+$) confirmed the simultaneous cleavage of 1 rhamnosyl and 1 glucosyl moiety. Signals for sugar fragments at m/z 326 ($[308 + \text{NH}_4]^+$) and 342 ($[324 + \text{NH}_4]^+$) 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 β -D-pyranosyl and α -L-pyranosyl configurations of the glucose, galactose, and rhamnose moieties were determined by ^1H - 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 $\text{MeCN}/\text{H}_2\text{O}$ 38:62 as eluent. The FAB-MS of **1b** and **1c** showed molecular ions at m/z 779 ($[M - \text{H}]^-$) indicating a mol. wt. for both compounds of 780. Signals at m/z 617 ($[(M - \text{H}) - 162]^-$) and 455

Table. ¹³C-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)} terminal	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
	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)} terminal	C(1)	102.5	102.5		102.6	102.6						
	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)} inner	C(1)							101.7	101.7	102.9	105.0 ^{g)}	
	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)} terminal	C(1)							101.7	101.8	102.0		
	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.

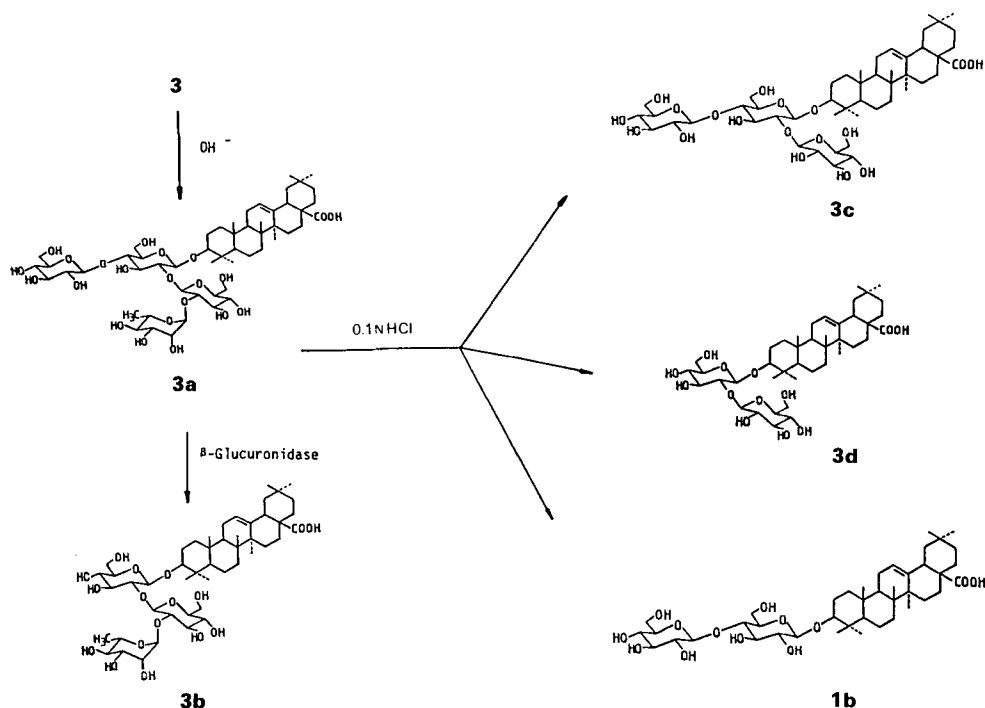
$[(M - H) - 324]^-$) corresponded to the subsequent loss of 2 hexosyl moieties. Acid hydrolysis of **1b** furnished glucose and oleanolic acid and of **1c** glucose, galactose, and oleanolic acid. The interglycosidic linkage in compound **1b** was determined by GC/MS of the partially methylated alditol acetates and by ¹³C-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 $\rightarrow$ 4)- β -D-glucopyranosyl]oleanolic acid, and consequently **1c** is 3-O-[O- β -D-galactopyranosyl-(1 $\rightarrow$ 3)- β -D-glucopyranosyl]oleanolic acid. Structure **1a** is, therefore, deduced to be 3-O-{O- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 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/H₂O 6:4 as eluent. Compound **2b** was identical (co-TLC, HPLC, ^{13}C -NMR) to 3-O-[O- β -D-glucopyranosyl-(1 $\rightarrow$ 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 $\rightarrow$ 3)-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 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 ($[M + \text{Cl}]^-$) and 925 ($[M - \text{H}]^-$) indicating a mol. wt. of 926. Additional signals at m/z 779 ($[(M - \text{H}) - 146]^-$), 617 ($[(M - \text{H}) - 308]^-$), and 455 ($[(M - \text{H}) - 470]^-$) 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 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl]oleanolic acid. This saponin was previously described in *Phytolacca dodecandra* as lemmatoxin C by *Parkhurst 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 $\text{MeOH}/\text{H}_2\text{O}$ 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 ($[(M + \text{NH}_4)]^+$). Addition signals were observed at m/z 798 ($[(M + \text{NH}_4) - 162]^+$) and 636 ($[(M + \text{NH}_4) - 324]^+$). Complementary fragments for **3** and **2** glucosyl units appeared at m/z 504 ($[486 + \text{NH}_4]^+$) and 342 ($[324 + \text{NH}_4]^+$) 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 $\rightarrow$ 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 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 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 Parkhurst *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 spermatozooids 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/H₂O [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.

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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 column (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 A1 sheets (Merck, Darmstadt) with CHCl₃/MeOH/H₂O 58:35:7 (system 1) or 70:30:3 (system 2) and RP-8-precoated glass plates (HPTLC, Merck, Darmstadt) with MeOH/H₂O 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. ¹H- and ¹³C-NMR: in (D₅)pyridine; Bruker WP-200 instrument at 200 and 50.29 MHz, resp.; for ¹³C-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 NH₃ 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-Pr)₂O/acetone 70:30. The aq. layer was adjusted to pH 6 with NaHCO₃. After evaporation to dryness, the sugars were extracted with pyridine from the residue and analysed by TLC on silica gel with AcOEt/MeOH/H₂O/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 H₂O-sat. BuOH (2 × 50 ml). The org. phase was washed with H₂O 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 H₂O-sat. BuOH (2 × 100 ml), washed with H₂O, 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 CH₃I (0.3 ml) for 90 min. The mixture was then extracted with Et₂O (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 with Na₂CO₃ 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 NaBD₄. 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 Ac₂O/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 (SiO₂, system 2): *R_f* 0.19. ¹³C-NMR (50.3 MHz, (D₅)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 (SiO₂, system 2): *R_f* 0.43. ¹³C-NMR (90.5 MHz, (D₅)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 (NH₃, positive ions): 960 [(*M* + NH₄)⁺], 798 [(*M* + NH₄) − 162]⁺, 636 [(*M* + NH₄) − 324]⁺, 474 [(*M* + NH₄) − 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 (SiO₂, system 2): *R_f* 0.75. ¹³C-NMR (101 MHz, (D₅)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-Glucopyranosyl 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 (SiO₂, system 2): *R_f* 0.1. ¹³C-NMR (90.5 MHz, (D₅)pyridine): δ's of the aglycone correspond to those described for bayogenin [7]; signals of 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 $\rightarrow$ 3)-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)]- β -D-glucopyranosyl]bayogenin (= 3 β -{[O- β -D-Galactopyranosyl-(1 $\rightarrow$ 3)-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)]- β -D-glucopyranosyl]oxy}-2 β ,23-dihydroxyolean-12-en-28-oic Acid; **2a**) was obtained from basic hydrolysis of **2**. Amorphous, white powder, 250–255° (dec.). TLC (SiO₂, system 2): *R_f* 0.29. ¹³C-NMR (90.5 MHz, (D₅)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 $\rightarrow$ 4)]- β -D-glucopyranosyl]bayogenin (= 3 β -[O- β -D-Glucopyranosyl-(1 $\rightarrow$ 4)]- β -D-glucopyranosyl]oxy]-2 β ,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 (SiO₂, system 2): *R_f* 0.62. ¹³C-NMR (101 MHz, (D₅)pyridine): chemical shifts of **2b** were identical to those of **1a** described in [7].

β -D-Glucopyranosyl 3-O-{O- α -L-Rhamnopyranosyl-(1 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)}-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)]- β -D-glucopyranosyl]oleanolate (= β -D-Glucopyranosyl 3 β -{[O- α -L-Rhamnopyranosyl-(1 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)]-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)]- β -D-glucopyranosyl]oxy}olean-12-en-28-oate; **3**). Amorphous, white powder, m.p. 254–258°. TLC (SiO₂, system 2): *R_f* 0.06. ¹³C-NMR (50.3 MHz, (D₅)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 (NH₃, positive ions): 1268 ([*M* + NH₄]⁺), 1106 ([(*M* + NH₄) – 162]⁺), 960 ([(*M* + NH₄) – 308]⁺), 944 ([(*M* + NH₄) – 324]⁺), 342 ([324 + NH₄]⁺), 326 ([308 + NH₄]⁺). Acid hydrolysis of **3** afforded oleanolic acid, D-glucose, and L-rhamnose.

3-O-{O- α -L-Rhamnopyranosyl-(1 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)]- β -D-glucopyranosyl]oleanolic Acid (= 3 β -{[O- α -L-Rhamnopyranosyl-(1 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 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 (SiO₂, system 2): *R_f* 0.14. ¹³C-NMR (90.5 MHz, (D₅)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 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranosyl]oleanolic Acid (= 3 β -{[O- α -L-Rhamnopyranosyl-(1 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 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 (SiO₂, system 2): *R_f* 0.42. ¹³C-NMR (50.3 MHz, (D₅)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 (= 3 β -[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 $\rightarrow$ 2)]- β -D-glucopyranosyl]oleanolic Acid (= 3 β -[O- β -D-Glucopyranosyl-(1 $\rightarrow$ 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 (SiO₂, system 2): *R_f* 0.74. ¹³C-NMR (50.3 MHz, (D₅)pyridine): chemical shifts of the aglycone correspond to those of **3a**; signals of the sugar moieties, see Table. DCI-MS (NH₃, positive ions): 798 ([*M* + NH₄]⁺), 636 ([(*M* + NH₄) – 162]⁺), 474 ([(*M* + NH₄) – 324]⁺).

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