PERGAMON

Phytochemistry 52 (1999) 1121–1131

PHYTOCHEMISTRY

New acylated triterpenoid saponins from *Maesa lanceolata*

Sandra Apers*, Tess E. De Bruyne, Magda Claeys, Arnold J. Vlietinck, Luc A.C. Pieters

Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium

Received 17 March 1999; received in revised form 17 May 1999; accepted 18 May 1999

Abstract

Ten new acylated triterpenoid saponins were isolated from the leaves of *Maesa lanceolata*. For their structure elucidation extensive use was made of homo- and heteronuclear 2D NMR techniques such as COSY, NOESY, HSQC and HMBC. All saponins identified contained the same tetraglycosidic side chain, but the triterpenoid moiety showed a variable esterification pattern. Monoester, diester and triester derivatives were present. Maesasaponin I was a 21-monoester derivative, i.e. {3β-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-13β,28-oxidoolean-16α, 22α, 28α-triol. Maesasaponins III, IV_3, V_3 and VI_2 had an additional acetyl, propanoyl, *n*-butanoyl and angeloyl substituent, respectively, in position 22. Maesasaponins II, IV_2, V_2, VI_3 and VII_1 were characterised as the 16-acetyl derivatives of maesasaponins I, III, IV_3, V_3 and VI_2, respectively. Structures of saponins previously reported in *M. lanceolata* had to be revised.

Keywords: *Maesa lanceolata*; Myrsinaceae; Acylated triterpenoid saponins; Maesasaponins; Structure elucidation

1. Introduction

Maesa lanceolata Forsskal. var. *golungensis* (Myrsinaceae), a shrub or small tree, occurs in many African countries, and is used in traditional medicine for various indications. We have already reported the characterisation of six acylated triterpenoid saponins in a biologically active saponin fraction from the leaves of this plant (Sindambiwe et al., 1996; Sindambiwe et al., 1998). Further chromatographic and spectroscopic work on this saponin fraction has resulted in the isolation and identification of a series of new acylated triterpenoid saponins, the structure elucidation of which is reported here, and has necessitated a revision of the previously reported structures.

2. Results and discussion

Repeated semi-preparative reversed-phase HPLC of a biologically active saponin-containing fraction of leaves of *M. lanceolata* afforded a series of new acylated triterpenoid saponins. All saponins identified (**1**–**10**) contained the same glycosyl moiety ([α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranoside) in position 3 of the aglycone, and the same oleane-derived triterpenoid (C_{30}) skeleton with hydroxyl substituents in positions 3, 16, 21 and 22, and a 13, 28-oxido moiety with a hemiacetal function at C-28 (13β, 28-oxidoolean-3β, 16α, 21β, 22α, 28α-pentol), the structures of which have been reported before (Sindambiwe et al., 1996). Compounds **1**–**10** showed a different esterification pattern at the C-16, C-21 and C-22 hydroxyl groups. Monoesters, diesters as well as triesters were found. The complete structure of all saponins reported here was confirmed by FABMS and extensive 1D and 2D NMR investigations, allowing complete ^1H and ^{13}C NMR assignments.

* Corresponding author. Tel.: +32-3-8202020; fax: +32-3-8202734.

E-mail address: sapers@uia.ua.ac.be (S. Apers).

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00352-0

FABMS of compound **1** (maesasaponin I) showed a $[M-H]^-$ ion at m/z 1233, indicating a M_r of 1234. This was in agreement with angeloyl substitution of the aglycone, which could be confirmed by ^1H and ^{13}C NMR spectroscopy. Signals characteristic for an angeloyl group occurred at δ 5.98 (*br q, J* = 7.2 Hz, H-3), 1.89 (*br d, J* = 7.2 Hz, H-4) and 1.85 (*br s*, H-5) in ^1H NMR, and at δ 167.27 (C-1), 128.63 (C-2), 135.40 (C-3), 20.59 (C-4) and 15.40 (C-5) in ^{13}C NMR. The site of esterification (C-21) was evident from the long-range C–H correlation between the carbonyl group of angelic acid and H-21 observed in a HMBC experiment. H-21 and H-22, correlated in a ^1H–^1H DQF-COSY experiment, formed a typical AB spin system. They showed direct C–H correlations in an HSQC experiment with ^{13}C NMR signals of C-21 and C-22, respectively. C-16 correlated in the HMBC experiment with H-28. Another relevant long-range correlation was observed between C-3 of the aglycone and glucuronic acid H-1. Detailed analysis of the 1D and 2D NMR (DQF-COSY, HSQC, HMBC) spectra allowed complete ^1H and ^{13}C NMR assignments, and the unequivocal establishment of the structure of maesasaponin I (**1**) as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-13β,28-oxidoolean-16α, 22α, 28α-triol. Characteristic ^1H NMR signals are listed in Table 1, and complete ^{13}C NMR assignments in Table 2.

FABMS of compound **2** (maesasaponin II) produced a $[M-H]^-$ ion at m/z 1275, corresponding to a M_r of 1276. Compared to **1**, this suggested an additional acetyl group. The presence of both an angeloyl and acetyl group was evident from ^1H and ^{13}C NMR (δ 2.10, *s* (H-2), and δ 168.92 and 21.59 (C-1 and C-2), respectively, for the acetyl group). A long-range C–H correlation of the angeloyl carbonyl group with H-21 observed in a HMBC experiment, showed that the angeloyl group occurred in position 21, as in maesasaponin I (**1**). Unfortunately no HMBC correlation occurred between the acetyl carbonyl group and the aglycone. The substitution pattern was elucidated based on the following observations: HMBC correlations of C-20, C-22, C-29 and C-30 with the ^1H NMR signal at δ 5.28 (*d, J* = 10.0 Hz) allowed to assign the latter to H-21. In addition H-21 showed a correlation with a ^1H NMR signal at δ 3.88, assigned to H-22, in the DQF-COSY experiment. C-17 showed a long-range C–H correlation with H-22 and a ^1H NMR signal at δ 5.21, assigned to H-16. ^1H NMR chemical shifts of H-16, H-21 and H-22 clearly indicated H-16, H-21 disubstitution, H-22 occurring more than 1.3 ppm to higher field (Jiménez et al., 1989; Aurada, Jurenitsch & Kubelka, 1984). In the HSQC spectrum the corresponding ^{13}C NMR signals were found at δ 67.52 (C-22), 79.75 (C-21) and 70.11 (C-16). Compared to **1**, esterification of the hydroxyl substituent in position 16 led to an upfield shift for C-21 and C-22 of 0.97 ppm and 1.47 ppm, respectively, and a downfield shift of 3.37 ppm for C-16. The structure of maesasaponin II (**2**) could unequivocally be established as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β-angeloyloxy-13β,28-oxidoolean-22α, 28α-diol. Characteristic ^1H NMR signals are listed in Table 1, and complete ^{13}C NMR assignments in Table 2.

The FABMS spectrum of maesasaponin III (**3**) showed a $[M-H]^-$ ion at m/z 1275 (M_r of 1276). ^1H and ^{13}C NMR spectroscopy revealed characteristic signals for an angeloyl and an acetyl group. Based on the long-range C–H correlations of angeloyl C-1 with H-21 and acetyl C-1 with H-22, the structure of maesasaponin III (**3**) was established as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-22α-acetoxy-21β-angeloyloxy)-13β,28-oxidoolean-16α, 28α-diol (^1H and ^{13}C NMR assignments, see Tables 1 and 2, respectively). Compared to maesasaponin I (**1**) esterification of the hydroxyl group in position 22 produced an upfield shift for C-21 of 2.56 ppm and a downfield shift for C-16 and C-22 of 0.66 and 2.15 ppm, respectively.

Whereas the structure elucidation of maesasaponin IV_1, which might contain a triterpenoid nucleus with a different hydroxylation pattern, is still in progress, the structures of maesasaponins IV_2 and IV_3 could be established. FABMS of maesasaponin IV_2 (**4**) produced a $[M-H]^-$ ion at m/z 1317, corresponding to a M_r of 1318, which is 42 amu higher than maesasaponin II (**2**), suggesting an additional acetyl group. Indeed, ^1H and ^{13}C NMR showed the presence of one angeloyl and two acetyl functionalities. The HMBC experiment provided proof for acetyl substitution at position 16 by showing a correlation between the carbonyl group of the acetyl substituent and H-16. The structure elucidation of such triesters posed an additional problem, since H-21 and H-22 had approximately the same chemical shift in ^1H NMR. Therefore, HMBC correlations of the carbonyl groups of the acyl moieties did not allow to establish their location in position 21 or 22. However, the esterification pattern could be unraveled by recording a NOESY spectrum and observing correlations between acyl substituents and the triterpenoid nucleus. NOESY correlations of H-28 with H-4 and H-5 but not with H-3 of the angeloyl group were observed. Construction of a model showed that these NOESY correlations can be expected in case of both a 21 (β)- and a 22 (α)- angeloyloxy substitution. No NOESY correlations occurred between H-28 and H-2 of an acetyl group. However, NOESY correlations did occur between H-28 and both

Table 1
Characteristic ^{1}H NMR assignments for maesasaponins **1**–**10** (δ) (*mult*., *J*) in DMSO-d_6 (400 MHz)

Proton no.	I (**1**)	II (**2**)	III (**3**)	IV_2 (**4**)	IV_3 (**5**)	V_2 (**6**)	V_3 (**7**)	VI_2 (**8**)	VI_3 (**9**)	VII_1 (**10**)
Aglycon										
3	2.96 (*br d*)	2.99 (*br d*)	2.94 (*br d*)	2.97 (*br d*)	2.98 (*br d*)	2.99 (*br d*, 11.1)	2.99 (*br d*, 10.6)	2.99 (*br d*, 11.3)	2.96 (*br d*)	2.96 (*br d*)
16	4.12 (*m*)	5.21 (*m*)	4.11 (*m*)	5.19 (*m*)	4.11 (*m*)	5.19 (*m*)	4.10 (*m*)	4.13 (*m*)	5.18 (*m*)	5.16 (*m*)
21	5.65 (*d*, 9.8)	5.28 (*d*, 10.0)	5.90 (*d*, 10.1)	5.41[a]	5.90 (*d*, 10.0)	5.43[a]	5.90 (*d*, 10.0)	5.95 (*d*, 10.0)	5.43[a]	5.52 (*d*, 10.3)
22	3.86[a]	3.88[a]	5.40 (*d*, 10.0)	5.41[a]	5.42 (*d*, 10.0)	5.43[a]	5.41 (*d*, 10.0)	5.50 (*d*, 10.0)	5.43[a]	5.52 (*d*, 10.3)
28	4.61 (*d*, 6.8)	4.74 (*br s*)	4.51 (*br s*)	4.70 (*br s*)	4.50 (*br s*)	4.70 (*br s*)	4.47 (*br s*)	4.52 (*br s*)	4.67 (*br s*)	4.70 (*d*, 5.6)
GlcA 1	4.38 (*d*, 7.0)	4.38 (*d*, 6.9)	4.38 (*d*, 7.1)	4.38 (*d*, 7.0)	4.41 (*d*, 7.0)	4.41 (*d*, 7.1)	4.37 (*d*, 7.2)	4.39 (*d*, 7.2)	4.37 (*d*, 7.0)	4.36 (*d*, 7.2)
Gal I 1	4.61 (*d*, 6.8)	4.61[a]	4.61 (*d*, 6.8)	4.61 (*d*, 7.0)	4.59[a]	4.60 (*d*, 7.1)	4.60[a]	4.62[a]	4.61 (*d*, 7.0)	4.61 (*d*, 7.1)
Gal II 1	5.03[a]	5.03[a]	5.02[a]	5.02[a]	5.01[a]	5.01[a]	5.03[a]	5.03[a]	5.03[a]	5.03[a]
Rha 1	5.06 (*br s*)[a]	5.03 (*br s*)[a]	5.02 (*br s*)[a]	5.05 (*br s*)[a]	5.03 (*br s*)[a]	5.03 (*br s*)[a]	5.05 (*br s*)[a]	5.03 (*br s*)[a]	5.05 (*br s*)[a]	5.03 (*br s*)[a]
Acyl (C16)										
2		2.10 (*s*)		2.15 (*s*)		2.16 (*s*)			2.16 (*s*)	2.15 (*s*)
Acyl (C21)										
3	5.98 (*br q*, 7.2)	6.02 (*br q*, 7.2)	6.01 (*br q*, 7.2)	6.07 (*br q*, 7.2)	6.02 (*br q*, 7.2)	6.08 (*br q*, 7.2)	6.03 (*br q*, 7.2)	5.99 (*br q*, 7.2)	6.09 (*br q*, 7.2)	6.05 (*br q*, 7.2)
4	1.89 (*br d*, 7.2)	1.89 (*br d*, 7.2)	1.86 (*br d*, 7.2)	1.87 (*br d*, 7.2)	1.86 (*br d*, 7.2)	1.87 (*br d*, 7.2)	1.87 (*br d*, 7.2)	1.84 (*br d*, 7.2)	1.88 (*br d*, 7.2)	1.83 (*br d*, 7.2)
5	1.85 (*br s*)	1.85 (*br s*)	1.77 (*br s*)	1.75 (*br s*)	1.76 (*br s*)	1.76 (*br s*)	1.77 (*br s*)	1.76 (*br s*)	1.76 (*br s*)	1.74 (*br s*)
Acyl (C22)										
2			2.10 (*s*)	2.15 (*s*)	a: 2.14 (*m*)	a: 2.04 (*m*)	a: 2.10 (*m*)		a: 2.03 (*m*)	
					b: 2.04 (*m*)	b: 2.04 (*m*)	b: 2.03 (*m*)		b: 2.00 (*m*)	
3					0.91 (*t*, 7.6)	0.88 (*t*, 7.7)	a: 1.43 (*m*)	5.90 (*br q*, 7.2)	a: 1.38 (*m*)	5.96 (*br q*, 7.2)
							b: 1.43 (*m*)		b: 1.38 (*m*)	
4							0.79 (*m*)	1.77 (*br d*, 7.2)	0.76 (*m*)	1.76 (*br d*, 7.2)
5								1.73 (*br s*)		1.64 (*br s*)

[a] No multiplicity and/or *J* value could be observed due to extensive overlap.

Table 2
^{13}C NMR assignments for maesasaponins **1**–**4** and **6**–**10** (δ) in DMSO-d_6 (100 MHz)

Carbon no.	I (**1**)	II (**2**)	III (**3**)	IV_2 (**4**)	V_2 (**6**)	V_3 (**7**)	VI_2 (**8**)	VI_3 (**9**)	VII_1 (**10**)
Aglycon									
1	38.60	38.57	38.63	38.58	38.55	38.64	38.61	38.59	38.58
2	25.70	25.63	25.72	25.64	25.64	25.65	25.63	25.69	25.68
3	89.11	89.05	89.12	89.12	89.02	89.06	89.01	89.10	89.10
4	38.88	38.87	38.92	38.87	38.83	38.90	38.85	39.09	38.87
5	54.87	54.82	54.86	54.83	54.80	54.87	54.83	54.84	54.83
6	17.19	17.07	17.17	17.09	17.06	17.17	17.13	17.11	17.11
7	33.50	33.45	33.50	33.42	33.41	33.51	33.47	33.41	33.41
8[a]	41.75	41.82	42.68	41.80	41.81	41.80	41.80	41.80	41.87
9	49.36	49.36	49.30	49.33	49.33	49.30	49.27	49.33	49.33
10	36.09	36.08	36.12	36.08	36.08	36.15	36.07	36.10	36.09
11	18.36	18.21	18.31	18.21	18.15	18.29	18.25	18.21	18.20
12	32.37	32.16	32.31	32.13	32.11	32.30	32.27	32.13	32.10
13	86.14	85.71	86.26	85.62	85.56	86.25	86.20	85.60	85.67
14[a]	42.85	42.52	42.83	42.25	42.21	42.65	42.63	42.20	42.27
15	35.08	31.82	35.10	31.95	31.99	35.14	35.13	32.03	31.93
16	66.75	70.11	67.41	70.06	70.06	67.52	67.84	70.11	70.61
17	53.28	52.69	52.30	52.15	52.16	52.75	52.78	52.20	52.18
18	44.79	44.90	45.01	44.19	44.14	44.98	44.99	44.19	44.30
19	37.55	37.33	37.18	36.90	36.90	37.18	37.17	36.89	36.93
20	36.30	36.14	36.52	36.36	36.34	36.55	36.54	36.45	36.47
21	80.71	79.75	78.14	77.60	77.58	78.08	78.14	77.50	77.52
22	68.99	67.52	71.14	70.72	70.55	71.73	71.57	70.45	70.20
23	27.35	27.32	27.38	27.35	27.32	27.38	27.32	27.35	27.35
24	15.80	15.77	15.84	15.81	15.93	15.82	15.76	15.81	15.81
25	16.02	15.97	16.03	16.00	15.93	15.99	15.94	16.32	16.00
26	18.11	18.01	18.09	18.02	17.95	18.05	18.01	18.01	18.01
27	18.79	18.95	18.93	19.00	18.94	18.90	18.92	19.01	19.07
28	96.38	95.69	95.67	94.95	94.93	95.60	95.69	95.03	95.01
29	29.81	29.79	29.35	29.31	29.25	29.34	29.30	29.33	29.34
30	20.43	20.05	20.82	19.76	20.21	20.29	20.21	19.78	19.74
GlcA									
1	103.80	103.78	103.82	103.85	103.76	103.74	103.73	103.84	103.84
2	78.29	78.29	78.31	78.30	78.26	78.40	78.30	78.33	78.39
3	80.10	80.11	80.13	80.11	80.26	80.19	80.23	80.10	80.06

4	69.81	69.78	69.82	69.81	69.68	69.75	69.74	69.84	69.87
5	75.03	74.95	75.09	75.08	75.07	74.88	75.00	75.04	74.94
6	170.48	170.48	170.47	170.50	170.22	170.40	170.44	170.60	169.06
Gal I									
1	102.04	102.00	102.07	102.06	102.05	102.03	102.02	102.04	102.02
2	71.12	71.11	71.14	71.14	71.13	71.07	71.13	71.15	71.16
3	73.41	73.40	73.44	73.43	73.42	73.44	73.41	73.44	73.44
4	67.86	67.84	67.88	67.88	67.86	67.76	67.84	67.88	67.88
5	74.60	74.57	74.61	74.62	74.56	74.57	74.56	74.62	74.62
6	60.25	60.23	60.28	60.29	60.25	60.26	60.22	60.28	60.27
Gal II									
1	99.48	99.43	99.52	99.50	99.93	99.46	99.48	99.48	99.44
2[b]	74.06	74.05	74.08	74.09	74.04	74.05	74.03	74.05	74.08
3[b]	74.06	74.05	74.08	74.09	74.15	74.20	74.13	74.09	74.08
4	69.28	69.25	69.32	69.31	69.25	69.25	69.23	69.30	69.28
5	75.18	75.14	75.23	75.21	75.21	75.14	75.16	75.04	75.15
6	60.45	60.41	60.50	60.48	60.43	60.44	60.40	60.46	60.44
Rha									
1	100.36	100.35	100.40	100.40	100.37	100.36	100.35	100.39	100.39
2	70.48	70.46	70.79	70.51	70.44	70.47	70.44	70.45	70.51
3	70.92	70.90	70.95	70.94	70.91	70.90	70.90	70.94	70.93
4	71.92	71.91	71.95	71.94	71.94	71.95	71.93	71.94	71.94
5	68.13	68.11	68.17	68.17	68.11	68.13	68.02	68.16	68.16
6	17.77	17.74	17.80	17.71	17.72	17.74	17.71	17.80	17.79
Acyl (C16)									
1		168.92		169.96	168.89	169.05	169.03		
2		21.59		21.56	21.44			21.56	21.50
Acyl (C21)									
1	167.27	167.26	166.70	166.88	166.70	166.50	166.44	166.90	166.74
2	128.63	128.30	128.69	127.33	127.24	127.80	127.72	127.20	127.41
3	135.40	136.15	136.73	137.68	137.83	137.15	136.78	138.29	137.82
4	15.40	15.50	15.34	15.48	15.38	15.31	15.20	15.52	15.22
5	20.59	20.56	20.21	20.30	19.68	20.20	20.18	20.34	20.24
Acyl (C22)									
1			168.76	168.44	171.47	171.20	165.85	170.90	164.98
2			21.40	21.56	27.18	35.75	128.23	35.69	127.26
3					8.85	18.03	135.19	17.74	137.64
4						13.36	15.13	13.39	15.42
5							20.15		20.09

[a,b] Assignments bearing the same superscript might be reversed.

Table 3
^{1}H and ^{13}C NMR assignments (δ), ^{1}H–^{1}H and long-range ^{13}C–^{1}H correlations for maesasaponin IV_3 (**5**) in DMSO-d_6

Position	^{13}C (δ)	^{1}H (δ, *mult.*, *J* (Hz))	^{1}H–^{1}H correlations	^{13}C–^{1}H correlations
Aglycon				
1	38.60	a: 1.60, *m*	H-1b	H-25
		b: 0.8, *m*	H-1a	
2	25.66	a: 1.71, *m*	H-2b, H-3	
		b: 1.52, *m*	H-2a, H-3	
3	89.05	2.98, *br d*	H-2a, H-2b	H-23, H-24, GlcA H-1
4	38.87			H-23, H-24
5	54.84	0.61, *d*, 10.6	H-6a, H-6b	H-23, H-24, H-25
6	17.14	a: 1.29, *m*	H-5	
		b: 1.38, *m*	H-5	
7	33.50	a: 1.40, *m*		H-26
		b: 1.06, *m*		
8[a]	41.81			H-26, H-27
9	49.29	1.06, *m*		H-25, H-26
10	36.09			H-25
11	18.27	a: 1.40, *m*	H-11b	
		b: 1.14, *m*	H-11a	
12	32.29	a: 1.90, *m*	H-12b	
		b: 1.25, *m*	H-12a	
13	86.20			H-27, H-28
14[a]	42.65			H-26, H-27
15	35.12	a: 1.81, *m*	H-15b, H-16	H-27
		b: 1.14, *m*	H-15a	
16	67.44	4.11, *m*	H-15a	H-22, H-28
17	52.70			H-22
18	44.97	1.71, *m*	H-19a	
19	37.18	a: 2.53, *m*	H-19b, H-18	H-29, H-30
		b: 1.22, *m*	H-19a	
20	36.47			H-21, H-29, H-30
21	78.14	5.90, *d*, 10.0	H-22	H-22, H-29, H-30
22	71.72	5.42, *d*, 10.0	H-21	H-21
23	27.35	0.95, *s*, (3H)		H-24
24	15.79	0.73, *s*, (3H)		H-5, H-23
25	15.96	0.79, *s*, (3H)		
26	18.03	1.06, *s*, (3H)		
27	18.88	1.16, *s*, (3H)		
28	95.63	4.50, *br s*[c]		H-22
29	29.30	0.82, *s*, (3H)		H-21, H-30
30	20.24	0.93, *s*, (3H)		H-29

GlcA				
1	103.74	4.41, *d*, 7.0	GlcA H-2	GlcA H-2, GlcA H-5
2	78.27	3.60, *m*	GlcA H-1, GlcA H-3	Gal I H-1
3	80.27	3.80, *t*, 8.7	GlcA H-2, GlcA H-4	GlcA H-2, GlcA H-4
4	69.68	3.35, *m*	GlcA H-3, GlcA H-5	GlcA H-3, GlcA H-5
5	75.07	3.66, *d*, 10.1	GlcA H-4	GlcA H-4
6	170.18			GlcA H-4, GlcA H-5
Gal I				
1	102.07	4.59[c]	Gal I H-2	GlcA H-2
2	71.12	3.25[c]	Gal I H-1, Gal I H-3	
3	73.41	3.22, *m*		
4	67.86	3.63, *m*	Gal I H-3	Gal I H-5
5	74.56	3.39, *m*		
6	60.25	3.35–3.50, *m*, (2H)		
Gal II				
1	99.56	5.01[c]	Gal II H-2	Gal II H-2, Gal II H-5, GlcA H-3
2[b]	74.05	3.49, *m*	Gal II H-1	
3[b]	74.15	3.49, *m*		
4	69.26	3.55, *m*		Gal II H-5
5	75.22	3.35, *m*		
6	60.45	3.35–3.50, *m*, (2H)		
Rha				
1	100.37	5.03[c]	Rha H-2, Rha H-3	Gal II H-2
2	70.44	3.66, *m*	Rha H-1, Rha H-3	Rha H-1
3	70.91	3.49, *m*	Rha H-1, Rha H-2, Rha H-4	Rha H-1
4	71.94	3.22, *m*	Rha H-3, Rha H-5	Rha H-6
5	68.11	3.85, *m*	Rha H-4, Rha H-6	Rha H-1, Rha H-6
6	17.73	1.10, *d*, 6.1, (3H)	Rha H-5	
angeloyl				
1	166.58			H-21, ang H-3, ang H-4, ang H-5
2	127.75			ang H-4, ang H-5
3	136.83	6.02, *br q*, 7.2	ang H-4	ang H-4, ang H-5
4	15.25	1.86, *br d*, 7.2, (3H)	ang H-3	ang H-3
5	20.16	1.76, *br s*, (3H)		ang H-3
propanoyl				
1	172.01			H-22, prop H-2a, prop H-2b, prop H-3
2	27.11	a: 2.14, *m*	prop H-3	prop H-3
		b: 2.04, *m*	prop H-3	
3	9.10	0.91, *t*, 7.6, (3H)	prop H-2a, prop H-2b	prop H-2a, prop H-2b

[a,b] Assignments bearing the same superscript might be reversed.

[c] No multiplicity and/or *J*-value could be observed due to extensive overlap.

H-16 and H-22, suggesting a β-configuration for the hydrogens and hence an α-configuration for the acetyl groups at C-16 and C-22. Therefore, the structure of maesasaponin IV_2 (**4**) could be established as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α, 22α-diacetoxy-21β-angeloyloxy)-13β,28-oxidoolean-28α-ol (^{1}H and ^{13}C NMR assignments, see Tables 1 and 2, respectively). Compared to maesasaponin II (**2**), a 16, 21-diester, esterification of C-22 led to an upfield shift of 2.15 ppm for C-21 and a downfield shift of 3.20 ppm for C-22. Compared to maesasaponin III (**3**), a 21, 22-diester, esterification of C-16 gave rise to an upfield shift of 0.54 and 0.42 ppm for C-21 and C-22, respectively, and a downfield shift of 2.65 ppm for C-16.

Maesasaponin IV_3 was the main component of the saponin fraction. FABMS indicated a M_r of 1290 ($[M-H]^-$ ion at m/z 1289). MS, ^{1}H and ^{13}C NMR indicated an angeloyl and a propanoyl (δ 2.14 (H-2a), 2.04 (H-2b) and 0.91 (H-3) in ^{1}H NMR, and δ 172.01 (C-1), 27.11 (C-2) and 9.10 (C-3) in ^{13}C NMR) group. Location of the angeloyl group at C-21 and the propanoyl group at C-22 was evident from 2D NMR spectra (HMBC). Hence, maesasaponin IV_3 (**5**) was characterised as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-22α-propanoyloxy-13β,28-oxidoolean-16α, 28α-diol (^{1}H and ^{13}C NMR assignments, see Tables 1 and 2, respectively).

The amount available for V_1 did not allow complete structure elucidation. FABMS, ^{1}H and ^{13}C NMR suggested that maesasaponin V_2 (**6**) was the acetyl derivative of maesasaponin IV_3 (**5**). Indeed, 2D NMR experiments including NOESY as described for **4**, confirmed its structure as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β-angeloyloxy-22α-propanoyloxy-13β,28-oxidoolean-28α-ol. Maesasaponin V_3 (**7**) was a homologue of maesasaponin IV_3, with a butanoyl instead of a propanoyl group at C-22, i.e. 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-22α-butanoyloxy-13β,28-oxidoolean-16α, 28α-diol (^{1}H and ^{13}C NMR assignments, see Tables 1 and 2, respectively).

Also the structure of maesasaponin VI_1 could not be established because of lack of material. In very much the similar way as described for other 21, 22-diesters such as **5** and **7**, maesasaponin VI_2 (**8**) was found to be a 21, 22-diangeloyl derivative, and characterised as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β, 22α-diangeloyloxy-13β,28-oxidoolean-16α, 28α-diol. Both compounds **9** and **10** were 16, 21, 22-triesters. Maesasaponin VI_3 (**9**) was the 16-acetyl derivative of maesasaponin V_3, i.e. 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β-angeloyloxy-22α-butanoyloxy-13β,28-oxidoolean-28α-ol. The acetyl group could be located at position 16 by HMBC, whereas NOESY correlations showed that the angeloyl group occurred in position 21, and the butanoyl group in position 22. Maesasaponin VII_1 (**10**) was the 16-acetyl derivative of maesasaponin VI_2 (**8**). Since the location of the acetyl group at C-16 was evident from HMBC, no NOESY experiment was necessary to establish its structure as 3β-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β, 22α-diangeloyloxy-13β,28-oxidoolean-28α-ol (^{1}H and ^{13}C NMR assignments, see Tables 1 and 2, respectively).

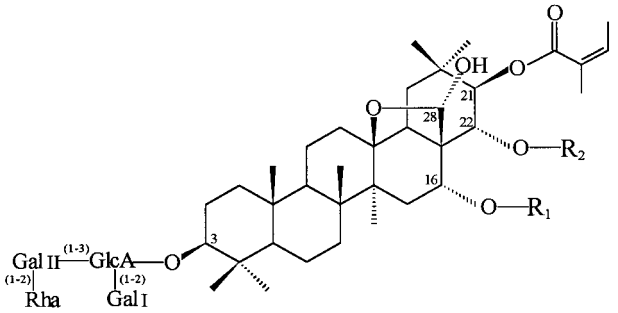
The relative stereochemistry of the saponin molecule, particularly at positions 3, 16, 21, 22 and 28, was established by correlations in NOESY spectra of mono-, di- and triesters. The NOESY cross peaks of H-22 with Me-30 and H-28 indicated the α-configuration of the hydroxyl or acyloxy group allocated at C-22. H-21 correlated with Me-29 and Me-30, but no NOESY correlations were observed between this proton and H-28 which is in agreement with a β-configuration of the hydroxyl or acyloxy group at position 21. The α-configuration of the C-16 substituent was evident from NOESY cross peaks between H-16 and H-28. All these correlations evidenced the α-configuration of the C-28 hydroxyl group. The ^{13}C NMR signal around δ 96, showing a significant glycosidation shift, was in addition to the previously mentioned HMBC evidence for **1** suggestive of the C-3 linkage of the sugar moiety. H-3 correlated with Me-23 and with H-5 of the aglycone nucleus in the NOESY spectrum, indicating the usual β-configuration of the C-3 hydroxyl group.

As an example, the complete ^{1}H and ^{13}C NMR assignments, as well as all correlations observed in the ^{1}H–^{1}H DQF-COSY, HSQC and HMBC spectra are listed in Table 3. Characteristic HMBC correlations are shown in Fig. 1.

TLC of the saponin mixture and all isolated compounds showed that no degradation or acyl migration had occurred during the chromatographic separation. Therefore it was obvious that the structures previously reported for the aglycones, i.e. the esterification pattern, had to be revised. After acid hydrolysis of the saponin mixture and column chromatography of the sapogenin mixture on silica gel, the aglycones had been characterised as 16, 22-diesters, with an angeloyl group in position 22, and a variable acyl group in position 16, e.g. 16-propanoyl (Sindambiwe et al., 1996). Since in the present work the angeloyl group was

unequivocally located at C-21, and, e.g. for maesasaponin IV_3, the propanoyl group at C-22, it appeared that under the conditions previously applied acyl migration might have occurred, at least in part, from C-22 to C-16 (various acyl residues) and from C-21 to C-22 (angeloyl residue). Acyl migrations between the 16α- and 22α-, as well as the 21β- and 22α-hydroxyl groups, have been described for related acylated saponins, for instance from *Aesculus hippocastanum* (Löw, 1967; Yosioka, Nishimura, Watani & Kitagawa, 1967; Wagner, Hoffmann & Löw, 1970).

In conclusion, the acylated triterpenoid saponins described above constitute a structurally consistent series of mono-, di- and triesters, in which C-21 is always substituted with an angeloyloxy group, C-16 can be substituted with an acetoxy group and C-22 with a variable acyloxy group (acetoxy, propanoyloxy, butanolyoxy or angeloyloxy).



	R_1	R_2		
Monoester	H	H	**1**	maesasaponin I
Diesters	acetyl	H	**2**	maesasaponin II
	H	acetyl	**3**	maesasaponin III
	H	propanoyl	**5**	maesasaponin IV_3
	H	butanoyl	**7**	maesasaponin V_3
	H	angeloyl	**8**	maesasaponin VI_2
Triesters	acetyl	acetyl	**4**	maesasaponin IV_2
	acetyl	propanoyl	**6**	maesasaponin V_2
	acetyl	butanoyl	**9**	maesasaponin VI_3
	acetyl	angeloyl	**10**	maesasaponin VII_1

3. Experimental

3.1. General experimental procedures

^1H, ^{13}C and 2D NMR spectra, including ^1H–^1H DQF-COSY, NOESY, HSQC (one-bond C–H correlations, indirectly detected) and HMBC (multiple-bond C–H correlations, indirectly detected) were recorded in DMSO-d_6 (99.9%) on a Bruker DRX-400 instrument operating at 400.15 MHz for ^1H, using standard software packages. HMBC experiments were optimised for a long-range C–H coupling constant of 8.3 Hz. In the NOESY experiments a mixing time ranging from 300 to 800 ms was used. Chemical shifts are quoted in δ units relative to TMS (0 ppm). FABMS spectra (−ion mode) were recorded on a Micromass VG70SEQ instrument, with glycerol as liquid matrix. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. TLC chromatography was carried out on RP-18 HPTLC-plates (Merck, Nano-DC, 0.2 mm layer thickness, 10 × 20 cm). The eluent consisted of H_2O–acetonitrile (1:1) containing 0.06% (v/v) trifluoroacetic acid. After spraying with *p*-anisaldehyde-sulphuric acid reagent and heating at 120°C for about 5 min, the spots were visualised.

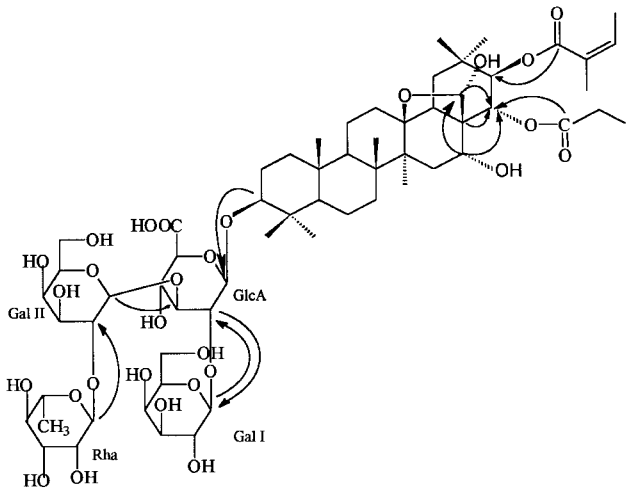


Fig. 1. Characteristic long-range ^{13}C–^1H correlations observed in a HMBC experiment for maesasaponin IV_3 (**5**).

3.2. Plant material

Leaves of *M. lanceolata* were collected in Butare, Rwanda in August 1989. The plant material was identified by Dr J. Mvukiyumwami of the botanical department of the IRST (Institut de la Recherche Scientifique et Technique), where a voucher specimen is kept.

3.3. Extraction and isolation

Extraction of the saponin fraction from dried and powdered leaves of *M. lanceolata* has been reported before (Sindambiwe et al., 1996), as well as the semi-preparative separation of this mixture by reversed-phase wide pore HPLC with temperature control (Apers, Foriers, Sindambiwe, Vlietinck & Pieters, 1998). Briefly, semi-preparative HPLC of 760 mg of the saponin mixture on a VYDAC® RP C_{18}, 5 μm, 300 Å (250 × 10 mm), using a gradient of 0.06% trifluoroacetic acid in H_2O (solvent A), and 0.06% trifluoroacetic acid in acetonitrile (solvent B) (38–56% B in 30 min) (flow rate: 5 ml/min), afforded seven fractions (I–VII). Fractions I–III consisted of chromatographically pure saponins, which were named maesasaponin

I (**1**, 8 mg), maesasaponin II (**2**, 10 mg) and maesasaponin III (**3**, 18 mg). Fractions IV (226 mg), V (143 mg), VI (89 mg) and VII (16 mg) were mixtures of saponins. Further semi-preparative HPLC on the same column using optimised chromatographic conditions afforded chromatographically pure subfractions. From 100 mg of fraction IV, maesasaponin IV_1 (28.3 mg), IV_2 (**4**, 14.7 mg) and IV_3 (**5**, 42.2 mg) were obtained; 80 mg of fraction V yielded maesasaponin V_1 (1.8 mg), V_2 (**6**, 27.8 mg) and V_3 (**7**, 22.3 mg). From 45 mg of fraction VI, maesasaponin VI_1 (2.2 mg), VI_2 (**8**, 16.2 mg) and VI_3 (**9**, 14.5 mg) were obtained; 14 mg of fraction VII afforded maesasaponin VII_1 (**10**, 7 mg) amd VII_2 (2.9 mg). Structures of compounds **1**–**10** were elucidated by FABMS and NMR spectroscopy, including the 2D NMR experiments mentioned previously.

*3.3.1. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-13β,28-oxidoolean-16α, 22α, 28α-triol (maesasaponin I, **1**)*

R_f value, 0.73, $[\alpha]_D^{31.4}$ −27.7° (*c* 0.84, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1233 $[M-H]^-$.

*3.3.2. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β-angeloyloxy-13β,28-oxidoolean-22α, 28α-diol (maesasaponin II, **2**)*

R_f value, 0.67, $[\alpha]_D^{32.5}$ −29.7° (*c* 0.69, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1275 $[M-H]^-$.

*3.3.3. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-22α-acetoxy-21β-angeloyloxy)-13β,28-oxidoolean-16α, 28α-diol (maesasaponin III, **3**)*

R_f value, 0.61, $[\alpha]_D^{33.7}$ −45.5° (*c* 1.00, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1275 $[M-H]^-$.

*3.3.4. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α, 22α-diacetoxy-21β-angeloyloxy)-13β,28-oxidoolean-28α-ol (maesasaponin IV_2, **4**)*

R_f value, 0.55, $[\alpha]_D^{35.0}$ −19.5° (*c* 1.00, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1317 $[M-H]^-$.

*3.3.5. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-22α-propanoyloxy-13β,28-oxidoolean-16α, 28α-diol (maesasaponin IV_3, **5**)*

R_f value, 0.46, $[\alpha]_D^{28.0}$ −41.5° (*c* 1.00, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1289 $[M-H]^-$.

*3.3.6. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β-angeloyloxy-22α-propanoyloxy-13β,28-oxidoolean-28α-ol (maesasaponin V_2, **6**)*

R_f value, 0.41, $[\alpha]_D^{29.5}$ −47.0° (*c* 1.00, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1331 $[M-H]^-$.

*3.3.7. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-22α-butanoyloxy-13β,28-oxidoolean-16α, 28α-diol (maesasaponin V_3, **7**)*

R_f value, 0.37, $[\alpha]_D^{32.0}$ −30.0° (*c* 0.50, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1303 $[M-H]^-$.

*3.3.8. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-21β, 22α-diangeloyloxy-13β,28-oxidoolean-16α, 28α-diol (maesasaponin VI_2, **8**)*

R_f value, 0.34, $[\alpha]_D^{33.0}$ −33.0° (*c* 1.00, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1315 $[M-H]^-$.

*3.3.9. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β-angeloyloxy-22α-butanoyloxy-13β,28-oxidoolean-28α-ol (maesasaponin VI_3, **9**)*

R_f value, 0.34, $[\alpha]_D^{34.0}$ −44.5° (*c* 1.00, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1345 $[M-H]^-$.

*3.3.10. 3β-O-{[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β, 22α-diangeloyloxy-13β,28-oxidoolean-28α-ol (maesasaponin VII_1, **10**)*

R_f value, 0.32, $[\alpha]_D^{34.5}$ −41.5° (*c* 0.59, pyridine). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2, respectively. FABMS (neg. ion mode) *m*/*z*: 1357 $[M-H]^-$.

Acknowledgements

This work was supported by the Fund for Scientific Research (FWO — Flanders, Belgium) (grant no. G.0119.96). MC (research director), TDB (post-doctoral researcher) and SA (research assistant) are associated with the FWO. This work was also supported as a Concerted Action by the Flemish Government (1999–2002).

References

Apers, S., Foriers, A., Sindambiwe, J. B., Vlietinck, A., & Pieters, L. (1998). Separation of a triterpenoid saponin mixture from *Maesa lanceolata*: semipreparative reversed-phase wide pore high performance liquid chromatography with temperature control. *Journal of Pharmaceutical and Biomedical Analysis*, *18*, 737–743.

Aurada, E., Jurenitsch, J., & Kubelka, W. (1984). Structure of triterpene-sapogenins of *Aesculus glabra, Planta Medica, 50*, 391–394.

Jiménez, M., Velásquez, K., Lira-Rocha, A., Ortega, A., Díaz, E., Aumelas, A., & Jankowski, K. (1989). Structure of a pentacyclic triterpenyl angelate from *Loeselia mexicana*. 1H 2D-NMR data and stereochemistry. *Canadian Journal of Chemistry*, *67*, 2071–2077.

Löw, I. (1967). Uber Acylwanderungen bei Protoäscigeninestern. *Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie*, *348*, 839–842.

Sindambiwe, J. B., Baldé, A. M., De Bruyne, T., Pieters, L., Van Den Heuvel, H., Claeys, M., Vanden Berghe, D. A., & Vlietinck, A. J. (1996). Triterpenoid saponins from *Maesa lanceolata*. *Phytochemistry*, *41*(1), 269–277.

Sindambiwe, J. B., Calomme, M., Geerts, S., Pieters, L., Vlietinck, A. J., & Vanden Berghe, D. A. (1998). Evaluation of biological activities of triterpenoid saponins from *Maesa lanceolata*. *Journal of Natural Products*, *61*, 585–590.

Wagner, J., Hoffmann, H., & Löw, I. (1970). Uber Inhaltstoffe des Rosskastaniensamens, VIII. Die Acylaglyka des Kryptoäescins und α-Ascins. *Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie*, *351*, 1133–1140.

Yosioka, I., Nishimura, T., Watani, N., & Kitagawa, I. (1967). On the unexpected identity of theasapogenol D (=camelliagenin A), a minor sapogneol of tea seeds, with dihydropriverogenin A. *Tetrahedron Letters*, *52*, 5343–5347.