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Diterpenoids from *Neoboutonia glabrescens* (Euphorbiaceae)

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Glabrescin, a daphnane diterpenoid, neoboutonin, a degraded diterpenoid with a novel skeleton, and neoglabrescins A and B, two rhamnofolane derivatives, have been isolated from the stem bark of *Neoboutonia glabrescens* Prain (Euphorbiaceae), together with the known tigliane derivative, baliospermin, and the known daphnane, montanin. Other constituents include squalene, 3-acetylaleuritolic acid, oleanolic acid and sitosterol, and the phenolic compounds 9-methoxy-1,7-dimethylphenanthrene and 2,3,8-tri-*O*-methylellagic acid. The structures were assigned on the basis of spectral studies and comparison with published literature data. The structures of neoglabrescins A and B were derived for their acetylated derivatives and, in the case of neoglabrescin A, confirmed by X-ray crystallographic analysis.

Keywords: *Neoboutonia glabrescens*; Euphorbiaceae; Glabrescin; Daphnanes; Neoboutonin; Rhamnofolanes; Neoglabrescins A and B

1. Introduction

The genus *Neoboutonia* (Euphorbiaceae) is widely distributed in tropical West Africa and represented by the species *N. diaguissensis* Beille, *N. manii* Benth, *N. glabrescens Prain* and *N. melleri* Prain var *vellutina* Prain. These species, with the exception of *N. diaguissensis*, grow in the anglophone part of Cameroon (Hutchison, 1958). The chemistry of this genus has not been extensively studied. However, tigliane derivatives and triterpenoids have been reported (Zhao et al., 1998) from the leaves of *N. melleri*. *N. glabrescens* Prain is a soft wooded tree of about 1.7 m height, which grows in open spaces in forests (Hutchison, 1958). It has skin irritant properties and is used in Cameroon ethnomedicine against worms, abdominal and stomach pains, and malaria (Thomas et al., 1989).

2. Results and discussion

In the course of our on-going research on Cameroonian medicinal plants used traditionally to treat human parasitic diseases (Tchuendem et al., 1999; Ayafor et al., 1994) we have studied the CH_2Cl_2–MeOH (1:1) extract of the stem bark of *Neoboutonia glabrescens* Prain. In addition to the known daphnane montanin (**2**), the known tigliane baliospermin (Ogura et al., 1978), 9-methoxy-1,7-dimethylphenanthrene (Long et al., 1997), 2,3,8-tri-*O*-methylmethylellagic acid (Nawwar et al., 1994; Yazaki and Hillis, 1976), 3-acetylaleuritolic acid (Woo and Hildebert, 1977; McLean et al., 1987), oleanolic acid, squalene, and sitosterol, two compounds glabrescin (**1**) and neoboutonin (**3**) were isolated. Acetylation of a polar fraction from the CH_2Cl_2–MeOH (1:1) extract of the stem bark with a mixture of pyridine-acetic anhydride afforded the acetates of two new rhamnofolane derivatives, neoglabrescins A (**4**) and B

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(**5**). The structure of neoglabrescin A was confirmed by X-ray crystallographic analysis.

Glabrescin was obtained as a yellow oil, $[\alpha]_D^{22} + 82.0°$ (*c* 0.35, $CHCl_3$). The molecular formula was deduced as $C_{48}H_{79}O_9$ from analysis of the ^{13}C NMR and DEPT data and the EI mass spectrum (*m*/*z* 798, $[M]^+$). The IR spectrum showed characteristic bands at 3465 (hydroxyl), 1763 (ester) and 1697 cm^{-1} (α,β-unsaturated cyclopentenone). The proton and carbon signals (Table 1), which were very close to those of the daphnane derivative montanin (**2**) (Ogura et al., 1978), were assigned unambiguously to the daphnane diterpenoid framework using a combination of ^1H–^1H COSY, HMQC and HMBC experiments. It was apparent that glabrescin contained an *ortho*-ester function (δ 119.8) and an ester attached to C-20. In the HMBC spectrum the ester carbonyl (δ 173.8) showed correlations to the characteristic AB proton pattern of 2H-20 [δ 3.85 (*d*, *J* = 11.9 Hz, H-20A) and 4.78 (*d*, *J* = 11.9 Hz, H-20B)]. Of particular note in the ^1H NMR spectrum were the resonances of two primary methyl groups (6H, δ 0.90, *t*, *J* = 7.0 Hz, Me-12′ and Me-16″), one doublet methyl (δ 1.18, *d*, *J* = 7.1 Hz, Me-18), two vinyl methyls [δ 1.80 (Me-17) and 1.82 (Me-19)], two methylene protons [δ 4.91 (H-16A) and 5.04 (H-16B)] and a deshielded olefinic proton [δ 7.61 (H-1)]. In addition to the methylene proton signals observed for 2H-12, integration identified 48 other methylene protons overlapping at δ 1.27–1.29 and 1.61–1.64 which could be assigned to the side chains. The presence of the ester carbonyl, the *ortho*-ester carbon and two primary methyl groups suggested the existence of two fatty chains. The mass fragments observed in the EI mass spectrum at *m*/*z* 183 and 239 could be assigned to lauroyl ($CH_3(CH_2)_{10}C{\equiv}O^+$) and palmitoyl ($CH_3(CH_2)_{14}C{\equiv}O^+$) ion fragments respectively. Daphnane derivatives with an *ortho*-ester involving a palmitoyl moiety have not yet been reported. In contrast, 20-palmitoyloxy daphnane diterpenoids are commonly found in the Euphorbiaceae (Kupchan et al., 1976; Adolph et al., 1984; Jolad et al., 1983). Since montanin (2) also occurs in this extract, it is reasonable to assume that glabrescin is montanin 20-palmitate (**1**).

Neoboutonin **3** was obtained as pale yellow crystals from MeOH–CH_2Cl_2. The EIMS showed a molecular ion peak at *m*/*z* 286 while ^1H- and ^{13}C-NMR spectra (Table 2) indicated the presence of sixteen non-exchangeable protons, two exchangeable protons and seventeen carbon atoms. This was consistent with the molecular formula $C_{17}H_{18}O_4$, whose nine double bonds equivalents could be accommodated by a ketone, a naphthalene ring system and an additional ring. The UV spectrum showed absorption maxima at λ_{max} 237 and 334 nm, corresponding to a conjugated aromatic system. The ^1H NMR spectrum of **3** revealed the presence of three methyl singlets at δ 1.12, 1.45 and 2.24

Table 1
NMR spectral data of glabrescin (**1**) and ^{13}C NMR spectral data of montanin (**2**) in $CDCl_3$

Carbon	**1**		**2**	
	δ_C (ppm)	δ_H (multiplicity, *J*)	HMBC correlations (H to C)	δ (ppm)
1	161.5	7.61	3, 4, 9, 19	161.7
2	136.9			137.0
3	210.1			210.3
4	72.6			72.6
5	70.3	4.28, *s*	3, 4, 6, 7, 10, 20	72.3
6	59.6			60.8
7	64.5	3.34, *s*	5, 6, 8, 9, 14, 20	64.7
8	36.9	2.92 (*d*, 2.5)	6, 7, 9, 10, 11, 13, 14	37.0
9	79.1			79.1
10	48.4	3.80[a]	2, 3, 5, 11	48.5
11	35.1	2.49, *m*	8, 9, 10, 13, 18	35.2
12	36.8	1.67/2.22 (*dd*, 14.3, 8.7)	9, 14, 15, 18	36.8
13	84.4			84.5
14	82.1	4.36 (*d*, 2.5)	7, 9, 9, 12, 13, 15	82.1
15	146.7			146.6
16	111.6	4.91/ 5.04, *s*	13, 15, 17	111.5
17	19.4	1.80, *s*	13, 15, 16	19.4
18	20.7	1.18 (*d*, 7.1)	9, 11, 12	20.7
19	10.3	1.82, *s*	1, 2, 3	10.3
20	66.2	3.85/4.78 (*d*, 11.9)	5, 6, 1	65.6
1′	119.8			119.7
2′	35.2	1.96/1.96[a]		35.2
12′	14.5	0.90[a]		14.5
1″	173.8			
2″	34.5	2.34 (*t*, 7.4)		
–$(CH_2)_n$–	23.1–32.3			23.1–32.3
16″	14.5	0.90[a]		

[a] Coupling constants not determined due to overlapping.

Table 2
^1H and ^{13}C NMR spectral data of neoboutonin (**3**)

Carbon	δ_C	δ_H	HMBC connectivities (H to C)
1	205.3		
3	64.6	4.06 (*s*)	1, 4, 18, 19
4	44.7		
5	167.6		
6	97.9	6.73 (*s*)	4, 5, 7, 8, 10
7	146.6		
8	119.6		
9	132.6		
10	120.9		
11	107.2	8.12 (*s*)	8, 10, 12, 13
12	159.2		
13	128.2		
14	125.5	7.87 (*s*)	7, 9, 12, 15
15	17.5	2.24 (*s*)	12, 13, 14
18	26.3	1.45 (*s*)	3, 4, 5, 19
19	26.9	1.12 (*s*)	3, 4, 5, 18
OMe	57.0	4.00 (*s*)	7

and a methoxyl group at δ 4.00. The substituents on the carbon skeleton were positioned using the correlations observed in the HMBC spectrum. Especially important were those between H-3 and C-1, C-10, C-18, and C-19, H-6 and C-5, C-7, C-8, and C-10, H-11 and C-10 and C-12 and H-14 and C-7 and C-12. The proposed structure 3 was further supported by the correlations observed in the NOE difference spectra. Irradiation of the methyl at δ 2.24 (Me-15) resulted in an increase of the intensity of H-14. The H-3 (δ 4.06) proton showed NOEs with both Me-18 (δ 1.45) and Me-19 (δ 1.12), that with the former being greater. The absolute configuration of the sole chiral center was not determined. It seems likely that neoboutonin (**3**), which has a novel carbon skeleton, is closely related to 1,7-dimethyl-9-methoxyphenanthrene (Long et al., 1997), another constituent of the extract, and that they both are isoprenoid in origin. The numbering system used for neoboutonin reflects its putative biogenetic origin. It is reasonable to assume that trigonostemone from *Trigostemon reidioides* (Euphorbiaceae) (Kokpol et al., 1990) and the phenanthrene derivatives from *Domohinea perrieri* (Euphorbiaceae) (Long et al., 1997) are also all degraded diterpenoids.

Acetylation of a polar fraction of the crude extract followed by chromatography afforded the acetates of two rhamnofolane derivatives neoglabrescins A (**4**) and B (**5**). Neoglabrescin A tetraacetate (**4a**) had a molecular formula $C_{25}H_{32}O_{11}$ as deduced from its EIMS, which showed a molecular ion peak at m/z 508. Its spectroscopic properties (Table 3) clearly indicated its trisnorditerpenoid nature. Its ^{13}C and DEPT spectra revealed seventeen skeletal carbons consisting of two methyl groups, three methylenes, seven methines and five quaternary carbon atoms, including a carbonyl group, a vinyl carbon and three oxygenated carbons. The compound was a tetraacetate as shown by the presence of four methyl singlets at δ 2.01, 2.08, 2.08 and 2.19 correlating to four ester carbonyls in the HMBC spectrum. The placement of three of these acetoxy groups was achieved using the HMBC correlations observed

Table 3
^1H and ^{13}C NMR spectral data of compound **4a** ($CDCl_3$) and **5a** (CD_3OD)

	4a			**5a**			
Carbon	δ_C	δ_H	Mult (J in Hz)	δ_C	δ_H	Mult (J in Hz)	HMBC (H→C)
1	124.2	5.50	*d* (1.6)	128.8	5.75	*s*	4.19
2	141.5	–		145.5	–		
3	77.3	5.85	*d* (1.4)	81.6	5.59	*s*	1, 2,–OAc
4	94.3	–		81.4	–		
5	78.4	6.34	*s*	83.3	5.03	*s*	7, 10,–OAc
6	88.7	–		84.2	–		
7	76.9	4.17	*d* (3.8)	79.2	4.08	*d* (10.7)	
8	45.8	2.36	overlapping	61.4	2.97	*dd* (10.8, 10.7)	
9	70.7	–		73.8	–		
10	55.8	2.95	*bs*	51.8	2.97	*bs*	
11	39.5	2.18	overlapping	42.8	2.39	*m*	
12a	45.8	2.29	overlapping	49.6	2.06	overlapping	
12b		2.45	*dd* (15.4, 12.2)		2.23	*dd* (16.3, 11.8)	
13	208.8	–		209.3	–		
14a	39.5	3.06	*t* (15.3)	58.8	3.20	overlapping	
14b		2.33	*dd* (15.5, 1.5)				
15	–			84.2	–		
16	–			30.5	1.24	*s*	15, 17
17	–			26.0	1.18	*s*	15, 16
18	14.3	1.00	*d* (6.6)	17.9	0.92	*d* (9.2)	9, 11, 12
19	13.6	1.67	*t* (1.3)	13.6	1.50	*s*	1, 2
20a	62.7	4.95	*d* (12.5)	64.9	4.65	*d* (11.8)	
20b		4.09	*d* (12.5)		4.31	d (11.8)	–OAc
CH_3CO–	170.8	–		173.5	–		
	170.7	–		172.8	–		
	170.5	–		172.2	–		
	170.0	–					
CH_3CO–	21.6	2.01	*s*	21.8	1.91	*s*	–OAc
	21.2	2.08	*s*	21.5	1.96	*s*	–OAc
	21.1	2.19	*s*	21.2	2.09	*s*	–OAc
	21.1	2.08	*s*				

Assignments are based on HMBC, HMQC and ^1H–^1H COSY experiments.

between H-3 (δ 5.85, *d*, *J* = 1.4 Hz), H-5 (δ 6.34, *s*), H-20a (δ 4.95, *d*, *J* = 12.5 Hz), H-20b (δ 4.09, *d*, *J* = 12.5 Hz) and three carbonyl esters. The IR spectrum showed a strong band at 3628 cm^{-1} corresponding to a hydroxyl group whose proton correlated in the HMBC spectrum with the carbon atom at δ 70.7 (C-9). These observations enabled us to attach the fourth acetoxy group at C-6 (δ 88.7). The vinyl proton (δ 5.50, *d*, *J* = 1.6 Hz, H-1) and methyl (δ 1.67, *t*, *J* = 1.3 Hz, Me-19) form part of the methylcyclopentene ring commonly found in diterpenoids from the Euphorbiaceae. The HMBC correlations observed between H-1 and C-2 (δ 141.5) as well as between Me-19 and C-1 (δ 124.2) further confirmed the presence of the double bond. The ^1H–^1H COSY spectrum delineated the partial connectivities H-7/H-8/2H-14 and Me-18/H-11/2H-12. The chemical shifts of H-12a (δ 2.29, *m*), H-12b (δ 2.45, *dd*, *J* = 15.4, 12.2 Hz), H-14a (δ 3.06, *t*, *J* = 15.3 Hz) and H-14b (δ 2.33, *dd*, *J* = 1.5, 15.5 Hz) showed that they were adjacent to a carbonyl function (δ 208.8, C-13). This was confirmed by the cross-peaks observed in the HMBC spectrum between H-14 and C-13. The secondary methyl group Me-18, a common feature of the cyclohexane ring of these derivatives appeared at δ_H 1.00 (*d*, *J* = 6.6 Hz). An uncommon feature was the presence of a C-4/C-7 ether linkage. HMBC correlations were observed between H-1, H-7 and C-4 (δ 94.3), enabling us to suggest the presence of this bridge. The relative stereochemistry of 1 was determined by NOE experiments. Irradiation of H-3 enhanced the intensities of H-5, H-10 and Me-19. NOEs were also observed between H-8, H-11 and H-12a, H-7, H-14b and H-20b as well as between H-5, H-3, H-10 and H-20a. The ether linkage was thus deduced to be β-oriented. Although rhamnofolane diterpenoids have been isolated from the Euphorbiaceae family (Stuart and Barrett, 1969; Jakupovic et al., 1988) this is the first time they have been found in the genus *Neoboutonia*. The structure of **4a** was confirmed by a single-crystal X-ray analysis (Fig. 1). Thus neoglabrescin A has the structure and stereochemistry shown in (**4**) and appears to have been derived by attack of a 4β-OH on a 6α,7α-epoxide precursor. The IR spectrum of the unacetylated mixture containing neoglabrescin A showed only ketonic carbonyl absorption, indicating that compound 4 had no esters present. Neoglabrescin A is a new trisnor-rhamnofolane derivative with an unusual 4,7-ether linkage. The loss of three carbons is readily explained by a retro-aldol reaction of the typical C-14 hydroxyisopropyl group of rhamnofolane derivatives.

The FABMS of neoglabrescin B triacetate (**5a**) displayed pseudomolecular ion peaks $[M+Na]^+$ and $[M+H]^+$ at *m*/*z* 547 and 525, respectively, consistent with the molecular formula $C_{26}H_{36}O_{11}$. Three acetoxy groups were identified in the ^1H NMR spectrum as methyl singlets at δ 1.91, 1.96 and 2.09 showing HMBC correlations with ester carbonyls at δ 172.2, 172.8 and 173.5. The twenty remaining carbon atoms, consisting of four methyl groups, two methylenes, eight methines and six quaternary carbons, were assigned to a rhamnofolane diterpenoid framework. Similarities were observed between the NMR spectral data of compound **5a** (Table 3) and those of **4a**, with the additional presence of an isopropyl group including two methyl singlets at δ 1.18 (Me-17) and 1.24 (Me-16) directly attached to a downfield oxygen-bearing carbon at δ 84.1 (C-15). The ^1H NMR and DEPT spectra showed that C-14 was a methine, bearing the isopropyloxy group. Moreover, the chemical shift of C-4 (δ 94.3) shifted to 81.4 ppm in **5a**, indicating the lack of esterification at this position. The large coupling constant (*J* = 10.7 Hz) between H-7 and H-8 showed that the two protons were *trans*. NOE interactions were observed between Me-16, H-14 and H-7, Me-17, H-20b and H-8 as well as between H-7, H-5 and H-14. These observations led to the conclusion that the isopropyloxy group was attached to C-7 through an ether linkage. The downfield chemical shift of C-15 (δ 84.2) was consistent with this conclusion. The ether ring was deduced to be β-oriented. HMBC correlations between the protons at δ 5.59 (*s*, H-3), 5.03 (*s*, H-5), 4.31 (*d*, *J* = 11.8, H-20b) and 4.65 (*d*, *J* = 11.8 Hz, H-20a) and the ester carbonyls showed that the acetates were attached at C-3, C-5 and C-20. The remaining carbons and protons were assigned by analysis of further ^1H, ^{13}C NMR, ^1H–^1H COSY, HMQC and HMBC data and by comparison with the NMR spectral data of **4a**. Thus neoglabrescin B (**5**) is a new rhamnofolane derivative which appears to have been derived by attack of a 15-OH on a 6α,7α-epoxide precursor.

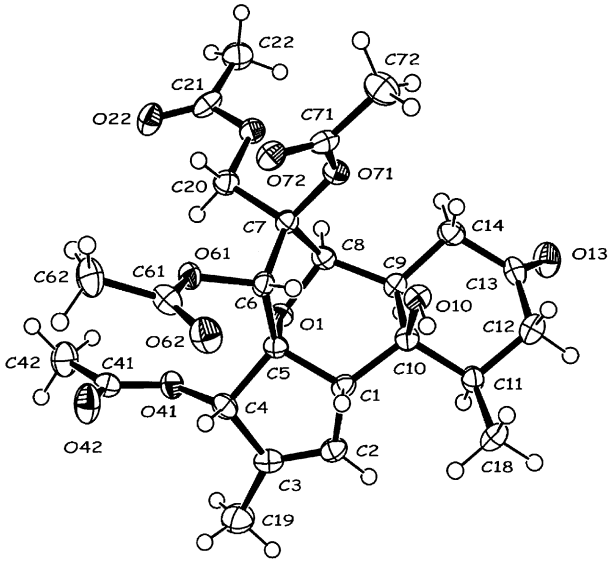
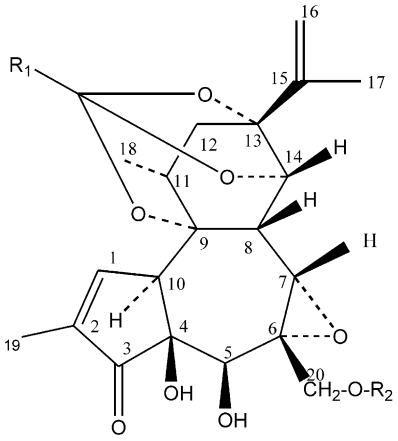
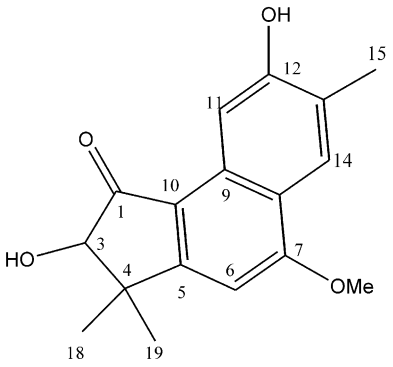


Fig. 1. *ORTEP* diagram of neoglabrescin A tetraacetate (**4a**).

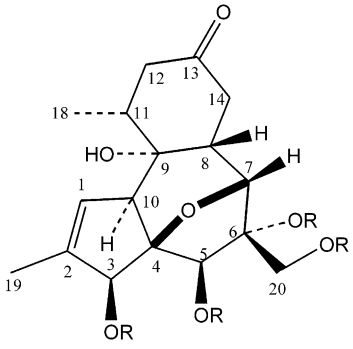


1 $R_1 = -\overset{2'}{CH_2}(CH_2)_9\overset{12'}{CH_3}$, $R_2 = -\overset{1''}{CO}-\overset{2''}{CH_2}-(CH_2)_{13}CH_3$

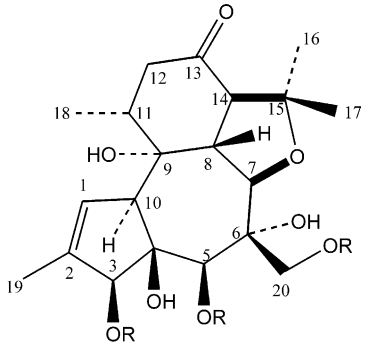
2 $R_1 = -\overset{2'}{CH_2}(CH_2)_9CH_3$, $R_2 = H$



3



4 R = H
4a R = Ac



5 R = H
5a R = Ac

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on an AA Series Automatic Polaar 2000 polarimeter. Melting points were determined by means of a Reitchert apparatus and are uncorrected. Mass spectra (70 eV) were recorded with a Jeol JMS 700 apparatus. The UV spectra were obtained with a Shimadzu 3101 PC instrument and the IR spectra determined with a Jasco FT-IR 410 apparatus. ^1H (400.6 MHz) and ^{13}C (100.13 MHz) Nmr spectra were recorded in $CDCl_3$ (with its signals at δ 7.25 and 77.0 ppm as standard reference) or in CD_3OD (with its signals at δ 3.21 and 49.4 ppm as standard reference) with a Brüker DPX 400 apparatus. NMR data acquisition and processing were performed with the aid of the XWIN NMR software package. NOE experiments were carried out using a Brüker AM 360 instrument. For MPLC, the chromatotron ser. no. 36B connected to a FMI pump QD (flow rate 10 ml/mn) was used with plates (2 mm) prepared with silica gel 60 PF_{254} containing $CaSO_4$. CC was run on Merck silica gel 60 and Sephadex LH-20, while TLC was carried out on silica gel 60 GF_{254} pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C.

3.2. Plant material

The stem bark of *Neoboutonia glabrescens,* Prain was collected at Mundemba (South-West, Cameroon) in July 1997. Mr. Paul Mezili, a retired botanist of the Cameroon National Herbarium, authenticated the plant material. Voucher specimens (BUD 0407) have been deposited at the Herbarium of the Botany Department of the University of Dschang.

3.3. Extraction and isolation

The dried and ground stem bark (2 kg) of *N. glabrescens* was extracted with a mixture of MeOH–CH_2Cl_2 (1:1) (4 l) to yield a crude organic extract (120 g) on drying. This extract was dissolved in MeOH–H_2O (1:4)

and extracted sequentially with CH_2Cl_2, EtOAc and *n*-BuOH. The combined CH_2Cl_2 and EtOAc extracts (68 g) were subjected to CC on Si gel, eluting with hexane-EtOAc followed by EtOAc–MeOH of increasing polarities to afford four main fractions A–D. Fraction A (1.5 g, eluted with EtOAc–hexane 1:4) gave squalene (10 mg) and sitosterol (14 mg). Fraction B (3 g, eluted with EtOAc–hexane 2:3) was passed over a Si gel column with CH_2Cl_2 as eluent to yield a sub-fraction which was further purified by gel permeation through Sephadex LH-20 [MeOH–CH_2Cl_2 (1:4)] to give glabrescin (**1**) (53 mg) as an orange oil and 3-β acetylaleuritolic acid (8 mg). Fraction C (3.5 g) eluted from the column with EtOAc–hexane (3:2) was separated on a Si gel column using mixtures of EtOAc–hexane of increasing polarity followed by repeated gel permeation chromatography through Sephadex LH-20 [CH_2Cl_2–hexane (1:4)] to give montanin (**2**) (700 mg), oleanolic acid (11 mg), baliospermin (50 mg), and a mixture which was purified by prep tlc [Me_2CO-CH_2Cl_2 (3:17)] to afford neoboutonin (3) (13 mg). Finally, fraction D (4 g, eluted with MeOH–EtOAc 1:9) was purified over a column with Me_2CO–CH_2Cl_2 (1:9) to give 2,3,8-tri-O-methylellagic acid (11 mg) and 9-methoxy-1,7-dimethylphenanthrene (14 mg) as a white powder.

The crude extract was passed through a silica gel column, eluting with hexane–EtOAc and EtOAc–MeOH mixtures of increasing polarity. The polar fraction (450 mg) obtained with EtOAc–MeOH (95:5) was treated with pyridine-acetic anhydride (1:1; 50 ml) and left overnight at rt. Concentration under reduced pressure yielded an acetylated mixture which was purified by MPLC using the chromatotron with CH_2Cl_2–MeOH of increasing polarity as eluent. The fraction obtained with 2% CH_2Cl_2–MeOH furnished neoglabrescin A tetraacetate (**4a**) (32 mg) while the 3% CH_2Cl_2–MeOH afforded neoglabrescin B triacetate (**5a**) (6 mg).

3.3.1. *Glabrescin (**1**)*

Orange oil; $[\alpha]_D^{24}$ +82 (*c* 0.35, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3465, 1736, 1697, 1458, 1378, 1159, 1115 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; EIMS (70 eV) *m/z* (rel. int.) $[M]^+$ 798 (6), 770 (11), 643 (1), 615 (2), 599 (5), 571 (10), 548 (2), 543 (9), 542 (6), 527 (17), 499 (13), 342 (32), 325 (70), 283 (47), 183 (35), 161 (33), 57 (100); anal. C 72.15%, H 9.82%, calc. for $C_{48}H_{79}O_9$, C 72.14%, H 9.84%.

3.3.2. *Neoboutonin (**3**)*

Pale yellow crystals (hexane–EtOAc); mp 277–278 °C; $[\alpha]_D^{20}$ −41 (*c* 0.2, MeOH); UV MeOH λ_{max} (log ε) 334 (3.3), 237 (2.8); IR ($CHCl_3$) ν_{max} 3430, 1628, 1105, 470 cm^{-1}; ^1H and ^{13}C NMR data see Table 2. EIMS (70 eV) *m/z* (rel. int.) $[M]^+$ 286 (80), 271 (100), 256 (50), 255 (65), 241 (50), 227 (12), 211 (12), 149 (20), 57 (20); anal. C 71.33%, H 6.33%, calc. for $C_{17}H_{18}O_4$, C 71.31%, H 6.34%.

3.3.3. *Neoglabrescin A tetraacetate (**4a**)*

Colorless crystals from acetone/petroleum ether; mp 247–248°; $[\alpha]_D^{20}$ −64.9° (*c* 0.7 $CHCl_3$); IR ν_{max} (KBr): 3628, 2977, 1745, 1515, 1422, 1363,1227, 1046 cm^{-1}; ^1H (400.6 MHz) and ^{13}C (100.13 MHz) NMR see Table 3; EIMS *m/z* 508 [M^+ $C_{25}H_{32}O_{11}$] (0.5), 466 (1), 448 (25), 406 (3), 388 (12), 328 (60), 286 (80), 268 (100), 263 (52), 221 (75), 163 (30), 150 (33), 108 (62).

3.3.4. *Neoglabrescin B triacetate (**5a**)*

Colorless crystals from acetone/petroleum ether; mp 215–216°; $[\alpha]_D^{20}$ +8.9 (*c* 0.09 MeOH); IR ν_{max} (KBr) 3439, 2969, 1743, 1689, 1631, 1371, 1260, 1060 cm^{-1}; ^1H and ^{13}C NMR see Table 3; FABMS *m/z* 547[M + Na]$^+$ (67), 525 $[M+H]^+$ (28), 524 [M^+,$C_{26}H_{36}O_{11}$, absent], 507 (3), 465 (2), 445 (3), 405 (2), 387 (3), 307 (9), 289 (13), 273 (6), 195 (6), 176 (9), 154 (100).

4. Experimental details of crystal structure determination

Details of data collection procedures and structure refinements are given in Table 4. A single crystal of suitable size was attached to a glass fibre using silicone grease, and mounted on a goniometer head in a general position. The crystal was cooled over a period of 0.5 h in the cold stream of the Oxford instruments Cryostream. Data were collected on a Enraf-Nonius KappaCCD diffractometer, running under Nonius collect software, and using graphite monochromated X-radiation (λ = 0.71073 Å) precise unit cell dimensions were determined by post-refinement of the setting angles of a significant portion of the data using Scalepack (Otwinowski and Minor, 1997). The frame images were integrated using Denzo (SMN) and resultant raw intensity files processed using a locally modified version of DENZOX. No absorption corrections were deemed necessary. Data were sorted and merged using SORTAV (Blessing, 1997). The structures were solved by direct methods using SIR-97. All non-H atoms were allowed under anisotropic thermal motion. C–H hydrogen atoms were included at calculated positions, with C–H = 0.96 Å, and were refined with a riding model and with U_{iso} set to 1.2 times of the attached C-atom. The O-H hydrogen atoms were found from difference maps and refined with a riding model. Refinement with SHELXL97-2 (Sheldrick, 1997) using full-matrix least-squares on F^2 and all the unique data and with the weighting scheme $w=[\sigma(F_o)^2+(AP)^2+BP]^{-1}$ where $P=[F_o^2/3+2F_c^2/3]$ and A = 0.0405, B = 0.1822 converged to the residuals shown in Table 4. The absolute configuration could not be determined experimentally from refinement of the Flack absolute parameter, and the known absolute configurations were assigned. Calculations using Platon indicated that there were no voids in

Table 4
Crystallographic data of **4a**

Compound formula	$C_{25}H_{32}O_{11}$
Compound color	Colorless
Mr	508.51
Space group	$P2_12_12_1$
crystal system	Orthorhombic
a/Å	8.9990 (3)
b/A	9.9599 (3)
c/Å	28.5395 (11)
V/Å^{-3}	2557.97 (15)
Z	4
D_{calc}/gcm^{-3}	1.32
F(000)	1080
μ(MoK_a)mm^{-1}	0.104
Temperature/K	100
Crystal size/mm	0.2×0.2×0.02
θ angle/deg	2.17–27.08
No. of data collected	9938
No. of unique data	5345
hkl range	−11→11;-12→12;-36→36
R_{int}	0.0514
No. of data in refinement	5345
No. of refined parameters	332
Final R[$I>2\sigma$(I)](all data)	0.0594 (0.1226)
R_w^2[$I>2\sigma$(I)](all data)	0.0975 (0.116)
Goodness of fit *S*	1.036
Flack absolute structure parameter	−0.9 (12)
Largest remaining feature in election density map/eA^{-3}	0.239–0.246
Max shift/esd in last cycle	0.001
$R = \Sigma(\lvert F_0\rvert - \lvert F_C\rvert)/\Sigma(F_o)$wR2 $= \left\{\Sigma\left(w\left(F_o^2 - F_c^2\right)^2\right)\right\}^{1/2}$	

$R_{int} = \Sigma\lvert F_o^2 - F_c^2(\text{mean})\rvert/\Sigma F_o^2$ (summation is carried out only where more than one symmetry equivalent is averaged).

the lattice capable of containing any solvent molecules. Thermal ellipsoids were obtained using the program ORTEP-3 for Windows (Farrugia, 1997). All calculations were carried out using the WinGX package of crystallographic programs (Farrugia, 1999).

Crystallographic data for structure **4a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-173033. Copies of the data can be obtained free of charge on application to CCDC, e-mail: deposit@ccdc.cam.ac.uk. Tables of observed and calculated structure factors are also available from L. J. Farrugia on request.

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