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CLERODANE-TYPE DITERPENES AND OTHER CONSTITUENTS OF *PENIANTHUS ZENKERI**

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Key Word Index—*Penianthus zenkeri*; Menispermaceae; alkaloids; clerodanes; 6-hydroxycolumbin; 6-hydroxyisocolumbin; penianthic acid methyl ester; 2β,3α-dihydroxy-2,3,7,8α-tetrahydropenianthic acid lactone; floribundic acid glucoside; zenkerin.

Abstract—Twenty-four compounds were isolated from *Penianthus zenkeri* and their structures determined by spectroscopic and chemical studies. 6-Hydroxycolumbin, 6-hydroxyisocolumbin, penianthic acid methyl ester, 2β-3α-dihydroxy-2,3,7,8α-tetrahydropenianthic acid lactone and the glycosides floribundic acid glucoside and zenkerin are new clerodanes or clerodane derivatives.

INTRODUCTION

Penianthus zenkeri grows in tropical West Africa as a small woody shrub up to a height of 1 m [2]; it is used in folk medicine against various diseases [2]. In the course of our investigations on West African medicinal plants [1] we started a phytochemical analysis of the roots in addition to the leaves, as there is only one short report on the existence of quarternary alkaloids in the roots of *P. zenkeri* [3].

RESULTS AND DISCUSSION

Air-dried plant material was extracted with petrol and then methanol, and the methanol extract distributed between water and dichloromethane and subsequently ethyl acetate. Repeated chromatography of these extracts and of the aqueous phase yielded five alkaloids, mostly of the protoberberine type, and 19 terpenoids (Table 1), whose structures were determined mainly by spectroscopic investigations. The clerodane-type diterpenes columbin and isocolumbin, besides β-amyrin and β-sitosterol, are the major constituents of *P. zenkeri*. Furthermore, the plant contains an array of other clerodane-type diterpenes, or corresponding glucosides in smaller amounts, among them the new compounds **14**, **16**–**18**, **21** and **24**.

The ^{1}H NMR spectra of columbin (**12**) and isocolumbin (**19**) differ characteristically by the chemical shifts and coupling constants of H-8, which is pseudo-equatorial in **12** but axial in **19**. This configurational situation is corroborated in the ^{13}C NMR by the typical shift of C-10; in **12** δ_{C-10} appears at higher field (*ca* −10 ppm) than in isocolumbin due to the neighbourhood of the axial C-17 carbonyl [4].

The structures of 6-hydroxycolumbin (**17**) and 6-hydroxyisocolumbin (**18**) were deduced from their NMR data (Table 2). COSY spectra and NOE enhancements observed between H-6 and Me-9 demonstrated the equatorial position of the 6-hydroxy group in both compounds. Consequently, **17** was epimerized by treatment with NaOH and the product was shown to be identical with **18**.

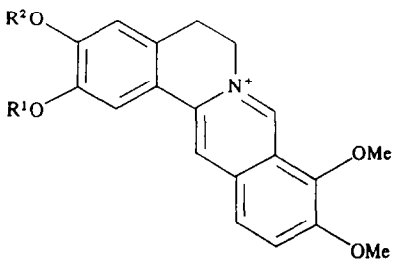
Heteronuclear COSY spectra and NOE experiments surprisingly revealed the hitherto unreported fact [5] that the ^{1}H signals of the methyl groups at C-5 and C-9 'change places' in the columbin and the isocolumbin series (Table 3).

Compound **21** was identified as the methyl ester of a new clerodane-type acid containing two non-conjugated double bonds, which we named penianthic acid. DCI mass spectrometry of the compound exhibited a quasi-molecular ion $[M+NH_4]^+$ at *m/z* 390 and its ^{1}H and ^{13}C NMR resembled major structural elements already known from columbin and isocolumbin. From the ^{1}H/^{1}H-COSY spectrum the double bonds were placed at Δ^2 and Δ^7; NOE studies established the positions of the methyl groups and the relative configurations at all steric centres (Fig. 1).

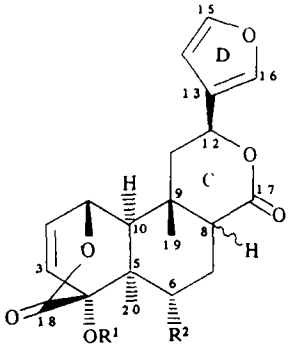
Similarly, the structure of **14** (=2β,3α-dihydroxy-2,3,7,8α-tetrahydropenianthic acid lactone) was established. In the ^{1}H and ^{13}C NMR all signals for the ring-systems B–D were observed as in isocolumbin. The ^{1}H/^{1}H-COSY and H/D-exchange experiments establish a secondary hydroxyl group at C-3, a tertiary hydroxyl group at C-4 and an acyloxy substituent at C-2, which according to elemental composition and IR should be part of a γ-lactone. The relative configuration again was determined by NOE studies (Fig. 2).

From the NMR spectra of the genuine compound and its tetraacetyl derivative, **16** was identified as the β-glucoside of **15**. The significant upfield shift of the anomeric carbon atom at δ95.9 is in agreement with an acylglucosidic structure [6, 7]. Consequently, **16** was cleaved with cellulase, or 2 M HCl–methanol, to yield floribundic acid (**15**) and glucose or a mixture of the methylglucosides, respectively. Trifluoroacetylation of

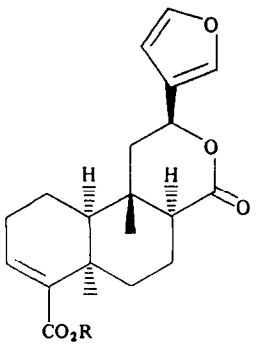
*Part 42 in the series 'Constituents of Tropical Medicinal Plants'. For Part 41 see ref. [1].



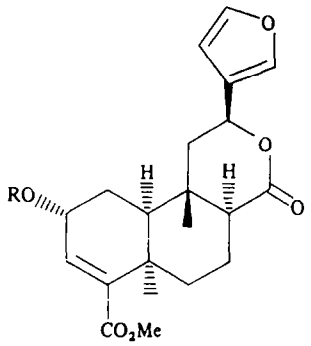
1 $R^1, R^2 = -CH_2-$
2 R^1 = Me, R^2 = H
4 $R^1 = R^2$ = Me



12 $R^1 = R^2$ = H, 8β-H
13 R^1 = Glc, R^2 = H, 8β-H
17 R^1 = H, R^2 = OH, 8β-H
18 R^1 = H, R^2 = OH, 8α-H
19 $R^1 = R^2$ = H, 8α-H
20 R^1 = Glc, R^2 = H, 8α-H



15 R = H
16 R = Glc
24 R = Xyl(1→6)Glc



22 R = H
23 R = Glc

the methylglucosides and subsequent GC analysis using a modified α-cyclodextrin [8] demonstrated that glucose belongs to the D-series.

Compound **24** [9] on acetylation gave the hexaacetyl derivative **25**. NMR studies of **24** and **25** established the structure of a floribundic acid ester with β-xylopyranosyl(1→6)-*O*-β-glucopyranose. Cleavage of **24** with HCl–methanol yielded a mixture of methylglucosides and methylxylosides, which were shown by GC analysis [8] to belong to the D-series.

Isocolumbin (**19**) has occasionally been discussed as an artefact produced by epimerization of columbin [10]. As our work-up of extracts was performed carefully and the compounds were exposed to only slightly acidic (silica gel) but not basic conditions, we regard **19** and **18** as genuine natural products.

Alkaloids of the protoberberine-type, among them **1**, **2** and **4**, are known to have antibacterial and antimycotic activity [11]. Therefore, the isolated major constituents were subjected to an antimycotic screening against *Botrytis cinerea*. In a plate diffusion test with filter discs (150 μg substance/disc) the alkaloids **1**, **2**, **4** and **5**, as well as the diterpenes **21** and **22**, showed positive effects by the production of inhibition zones ≥ 8 mm.

Table 1. Constituents of *Penianthus zenkeri*

Classification	Compound	Extract	Concentration*
Alkaloids	Berberine (**1**)	M	0.1
	Jatrorrhizine (**2**)	M	0.1
	Menisperine (**3**)	M	0.1
	Palmatine (**4**)	M	0.1
	trans-Feruloyltyramine (**5**)	M	0.04
Terpenoids			
Triterpenes	β-Amyrin (**6**)	P	0.5
	2α,3β-Dihydroxyolean-12-ene (**7**)	P	0.2
	2α,3β,28-Trihydroxyolean-12-ene (**8**)	P	0.01
Sterols	20-Hydroxyecdysone (**9**)	M	0.2
	β-Sitosterol (**10**)	P	0.5
	β-Sitosterol glucoside (**11**)	M	0.1
Diterpenes	Columbin (**12**)	M	2
	Columbin glucoside (**13**)	M	0.2
	2β,3α-Dihydroxy-2,3,7,8α-tetrahydropenianthic acid lactone (**14**)	M	0.01
	Floribundic acid (**15**)	M	0.02
	Floribundic acid glucoside (**16**)	M	0.01
	6-Hydroxycolumbin (**17**)	M	0.01
	6-Hydroxyisocolumbin (**18**)	M	0.01
	Isocolumbin (**19**)	M	1
	Isocolumbin glucoside (**20**)	M	0.04
	Penianthic acid methyl ester (**21**)	M	0.05
	Tinophyllol (**22**)	M	0.01
	Tinophylloloside (**23**)	M	0.02
	Zenkerin (**24**)	M	0.03

*Estimated concentrations in % of petrol (P) and MeOH (M) extracts, respectively.

Table 2. ^{1}H NMR data of compounds **12**, **19**, **17** and **18** (CD_3OD, 400 MHz)

H	**12**	**19**	**17**	**18**
1	5.30 *br d* (5)*	5.25 *br d* (5)	5.25 *dd* (5, 1.8)	5.23 *dd* (5.5, 1.8)
2	6.54 *dd* (8, 5)	6.54 *dd* (8, 5)	6.58 *dd* (8, 5)	6.57 *dd* (8, 5.5)
3	6.27 *dd* (8, 1.8)	6.26 *dd* (8, 1.8)	6.22 *dd* (8, 1.8)	6.22 *dd* (8, 1.8)
6	1.73 *br dd* (14.5, 8)		4.20 *dd* (10.2, 8)	4.16 *dd* (8.5, 8.5)
	1.41–1.51 *m*	1.36–1.46 *m*		
7	2.03–2.13 *m*	2.06–2.14 *m*	2.46 *ddd* (15, 10.2, 2.2)	2.53 *ddd* (14, 9.5, 8.5)
	2.52–2.63 *m*		2.33 *ddd* (15, 11.2, 8)	1.69 *ddd* (14, 8.5, 8.5)
8	2.57 *dd* (11.8, 2)	2.98 *dd* (10.5, 8)	2.74 *dd* (11.2, 2.2)	3.20 *dd* (~9, ~9)
10	1.84 *s*	1.86 *s*	2.03 *s*	2.05 *s*
11	1.98 *dd* (14.9, 12.2)	1.92 *dd* (14.5, 12.1)	1.96 *dd* (15, 12.2)	1.92 *dd* (14.5, 12.5)
	2.39 *dd* (14.9, 4.2)	2.30 *dd* (14.5, 3.5)	2.36 *dd* (15, 4)	2.30 *dd* (14.5, 4)
12	5.59 *dd* (12.2, 4.2)	5.56 *dd* (12.1, 3.5)	5.58 *dd* (12.2, 4)	5.56 *dd* (12.5, 4)
14	6.56 *m*	6.54 *m*	6.56 *m*	6.53 *m*
15	7.51 *m*	7.51 *m*	7.51 *m*	7.51 *m*
16	7.61 *m*	7.60 *m*	7.61 *m*	7.59 *m*
Me-5	1.00 *s* (3H)	1.20 *s* (3H)	1.05 *s* (3H)	1.26 *s* (3H)
Me-9	1.22 *s* (3H)	1.02 *s* (3H)	1.24 *s* (3H)	1.04 *s* (3H)

*Coupling constant (Hz)

EXPERIMENTAL

General. Analytical TLC was performed on precoated plates (Nano plates Sil-20 UV, Machery-Nagel) using S-1: $CHCl_3$–MeOH (9:1), S-2: $CHCl_3$–MeOH (4:1), S-3: $CHCl_3$–MeOH (19:1), S-4: EtOAc–MeOH (9:1), S-5: EtOAc–MeOH (7:3), S-6: cyclohexane–Me_2CO (4:1) and S-7: petrol–Me_2CO (9:1). Detection: anisaldehyde reagent [12]. Unless otherwise indicated, MS were recorded by EIMS (70 eV). Unless otherwise stated, ^{1}H NMR were measured at 400 MHz and ^{13}C NMR at 100 MHz with TMS as int. standard.

Plant material. Roots of *P. zenkeri* Engl. & Diels were collected in 1984 in Ghana and identified by Mr A. A. Enti (Forestry

Table 3. ^{1}H resonances of methyl groups at C-5 and C-9 in the columbin and isocolumbin series (in CD_3OD)

	δ_{Me}	
	Me-5	Me-9
Columbin (**12**)	1.00	1.22
6-Hydroxycolumbin (**17**)	1.05	1.24
Isocolumbin (**19**)	1.20	1.02
6-Hydroxyisocolumbin (**18**)	1.26	1.04

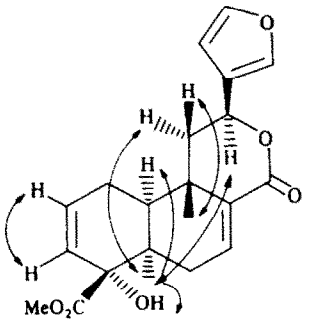


Fig. 1. Structure of **21**, and NOE enhancements observed in ^{1}H NMR.

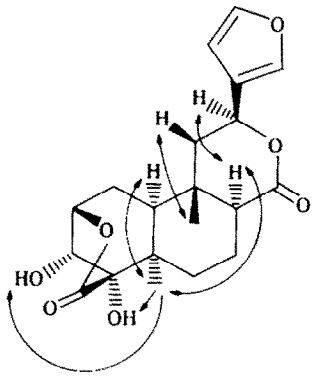


Fig. 2. Structure of **14**, and NOE enhancements observed in ^{1}H NMR.

Enterprises, Legon, Ghana). A voucher specimen (No. 84-06) is deposited in our institute in Erlangen.

Extraction and isolation. Roots (1.8 kg) were extracted successively with petrol and then MeOH at room temp. yielding 7 g and 120 g of crude extracts, respectively. The MeOH extract (100 g) was redissolved in 400 ml MeOH, dil. with 400 ml H_2O and successively extracted with CH_2Cl_2 (4.5 g residue, Ext. A) and then with EtOAc (3.5 g residue, Ext. B).

Subsequently Mayer's reagent was added to the aq. layer, the ppt. filtered off and redissolved in Me_2CO–H_2O (1:1). This soln was treated with Amberlite® IRA 400 (Cl^- form) (12 hr, room temp. with stirring), filtered and evapd. CC on Alox N with $CHCl_3$–MeOH (9:1) yielded the quarternary alkaloids **1**–**4**.

The petrol extract (5 g) was subjected to CC on 300 g silica gel (Macherey-Nagel, No. 81538) using petrol and petrol–Me_2CO mixts to yield **6**–**8** and **10**.

Extract A was subjected to CC on 350 g silica gel (Macherey-Nagel) using $CHCl_3$ and $CHCl_3$–MeOH mixts. The resulting frs were rechromatographed repeatedly on 100-fold amounts of silica gel using cyclohexane–EtOAc and/or on PVA-500 (Merck) using EtOAc–MeOH to yield **5**, **11** and the diterpenes **12**, **14**, **15**, **17**–**19**, **21** and **22**. HPLC of some of the more polar frs on silica gel RP-18 with MeOH–H_2O (1:1) resulted in the isolation of the glycosides **16** and **23**. The most polar fr. contained the same quarternary alkaloids **1**–**4** as the aq. layer of Ext. B.

Ext. B was also sepd by CC on silica gel using EtOAc–MeOH (19:1) and the resulting frs subjected to CC on PVA-500 using MeOH and then silica gel using $CHCl_3$–MeOH–H_2O (50:10:1) to yield in the least polar fr., glycosides **13** and **20**, and in the more polar fr. **9**. Isolation of **24** was achieved by HPLC of the most polar fr. on silica gel RP-18 using MeOH–H_2O (1:1).

Berberine (**1**). Yellow, amorphous (10 mg). TLC: R_f 0.32 (S-2); UV/Vis, ^{1}H NMR, MS in agreement with published data [13].

Jatrorrhizine (**2**). Orange, amorphous (9 mg). TLC: R_f 0.23 (S-2), UV/Vis, ^{1}H NMR, MS in agreement with published data [13].

Menisperine (**3**). Amorphous (16 mg). TLC: R_f 0.17 (S-2). $[\alpha]_D$ +136° (MeOH; *c* 0.3). UV, IR, ^{1}H NMR in agreement with published data [14].

Palmatine (**4**). Yellow, amorphous (62 mg). TLC: R_f 0.31(S-2). UV/Vis, MS in agreement with published data [13].

trans-*Feruloyltyramine* (**5**). Oil (12 mg). TLC: R_f 0.61 (S-2). UV, ^{1}H NMR, ^{13}C NMR, MS in agreement with published data [15].

β-Amyrin (**6**). Crystals (11 mg). Mp 198°. TLC: R_f 0.52 (S-6). $[\alpha]_D$ +88° ($CHCl_3$; *c* 1.1). ^{1}H NMR, ^{13}C NMR, MS identical with an authentic sample.

2α,3β-Dihydroxyolean-12-ene (**7**). Crystals (7 mg). Mp 196–197° (ref. [16] mp 198–203°). TLC: R_f 0.27 (S-6). $[\alpha]_D$ +69° ($CHCl_3$; *c* 0.4) (ref. [16] $[\alpha]_D$ +79.9° ($CHCl_3$)). ^{1}H NMR in agreement with published data [17].

2α,3β,28-Trihydroxyolean-12-ene (**8**). Crystals (5 mg). Mp 273°. TLC: R_f 0.13 (S-6). $[\alpha]_D$ +64° ($CHCl_3$; *c* 0.4). IR, ^{13}C NMR in agreement with published data [18, 19].

20-Hydroxyecdysone (**9**). Crystals (100 mg). Mp 236–238°. TLC: R_f 0.28 (S-2). $[\alpha]_D$ +68° (MeOH; *c* 0.85). UV, IR, ^{1}H NMR in agreement with published data [20].

β-Sitosterol (**10**). Crystals (9 mg). Mp 137–138°. TLC: R_f 0.18 (S-7). $[\alpha]_D$ −36° ($CHCl_3$; *c* 1.1). IR, ^{1}H NMR, MS identical with an authentic sample.

β-Sitosterol glucoside (**11**). Amorphous (10 mg). Mp 286° (dec.). TLC: R_f 0.26 (S-2). $[\alpha]_D$ −36° ($CHCl_3$; *c* 0.8). ^{13}C NMR in agreement with published data [21].

Columbin (**12**). Crystals from MeOH (26 mg). Mp 183° (lit. [22] mp 182°). TLC: R_f 0.59 (S-3); anisaldehyde reagent: blue. $[\alpha]_D$ +35° (MeOH; *c* 0.1) (lit. [23] $[\alpha]_D$ +38° (MeOH)). MS *m/z* (rel. int.): 358.1416 ($=C_{20}H_{22}O_6$) $[M]^+$ (4), 314 $[M-CO_2]^+$ (8), 247 (17), 246 (39), 231 (46), 153 (80), 152 (81), 121 (31), 112 (31), 109 (39), 108 (70), 107 (100).

Columbin glucoside (**13**). Amorphous (25 mg). TLC: R_f 0.38 (S-2); anisaldehyde reagent: blue. $[\alpha]_D$ +37° (pyridine; *c* 1.0) (lit. [24] $[\alpha]_D$ +28.9° (pyridine, *c* 1.49)). IR, ^{1}H and ^{13}C NMR in agreement with published data [24].

2β,3α-Dihydroxy-2,3,7,8-tetrahydropenianthic acid lactone (**14**). Crystals (4 mg). Mp 221–223°. TLC: R_f 0.64 (S-2); anisaldehyde reagent: blue-violet. $[\alpha]_D$ +5° (MeOH; *c* 0.35). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1780, 1745, 1600. ^{1}H NMR (DMSO-d_6): δ 0.83 (3H, *s*, Me-9), 1.38 (3H, *s*, Me-5), 1.52–1.76 (5H, *m*, H-1, H-6, H-7, H-11$_{ax}$), 1.94 2.00 (1H, *m*), 2.03 (1H, *dd*, J_1 = 14.5, J_2 = 4 Hz, H-11$_{eq}$), 2.16 2.26 (2H, *m*, H-1, H-10), 2.96 (1H, *dd*, $J_1 \sim J_2 \sim 9$ Hz, H-8), 4.15 (1H, *dd*, J_1 = 6, J_2 = 4.5 Hz, H-3), 4.55 (1H, *m*, H-2), 5.53 (1H, *dd*, J_1 = 12, J_2 = 4 Hz, H-12), 5.57 (1H, *s*, exchangeable, OH-4), 6.04 (1H, *d*, J = 4.5 Hz, exchangeable, OH-3), 6.57 (1H, *m*, H-14), 7.66 (1H, *m*) and 7.70 (1H, *br s*) (H-15 and H-16). ^{13}C NMR (CD_3OD): δ 16.8, 22.9, 24.8, 26.6, 27.8, 38.9, 41.5, 42.9, 47.4, 49.3,

71.2, 76.2, 78.6, 79.9, 109.7, 125.8, 141.4, 144.9, 177.7, 178.8. MS *m/z* (rel. int.): 376.1523 (=$C_{20}H_{24}O_7$) $[M]^+$ (21), 247, (17), 133 (16), 121 (46), 107 (33), 95 (43), 94 (100).

Floribundic acid (**15**). Amorphous (8 mg). TLC: R_f 0.45 (S-1); anisaldehyde reagent: violet. $[\alpha]_D$ −122° ($CHCl_3$; *c* 0.4) (lit. [25] $[\alpha]_D$ −130° ($CHCl_3$)). IR, ^{13}C NMR and MS in agreement with published data [25, 26].

Floribundic acid glucoside (**16**). Amorphous (7 mg). TLC: R_f 0.49 (S-2); anisaldehyde reagent: violet. $[\alpha]_D$ −60° (MeOH; *c* 0.75). IR ν_{max}^{KBr} cm^{-1}: 3430, 1720, 1630. ^1H NMR (CD_3OD): δ 1.03 (3H, *s*, Me-9), 1.29 (3H, *s*, Me-5), 1.2–1.35 (1H, *m*), 1.39–1.50 (1H, *m*), 1.68–1.76 (2H, *m*), 1.84–1.92 (1H, *m*), 1.97 (1H, *dd*, J_1 = 14, J_2 = 11.5 Hz, H-11_{ax}), 2.13 (1H, *dd*, J_1 = 14, J_2 = 6 Hz, H-11_{eq}), 2.15–2.50 (3H, *m*), 2.78 (1H, *dd*, J_1 = 11.5, J_2 ~ 2.5 Hz, H-8), 2.88 (1H, *ddd*, J_1 = 14, $J_2 \sim J_3 \sim 3$ Hz, H-6), 3.33–3.87 (6H, sugar protons), 5.50 (1H, *dd*, J_1 = 11.5, J_2 = 6 Hz, H-12), 5.56 (1H, *d*, *J* = 8 Hz, H-1'), 6.50 (1H, *m*, H-14), 6.80 (1H, *dd*, $J_1 \sim J_2 \sim 3.5$ Hz, H-3), 7.50 (1H, *m*) and 7.58 (1H, *m*) (H-15 and H-16). ^{13}C NMR (pyridine-d_5): δ 17.4 (C-1, C-2 or C-7), 20.6 (C-7, C-1 or C-2), 22.0 (C-19), 23.7 (C-2, C-7 or C-1), 32.9 (C-20), 35.7 (C-6), 36.5 (C-9), 38.3 (C-5), 45.6 (C-11), 49.1 (C-8), 51.9 (C-10), 62.3 (C-6'), 70.2 (C-12), 71.2 (C-4'), 74.2 (C-2'), 78.8 (C-5'), 79.6 (C-3'), 95.9 (C-1'), 109.7 (C-14), 125.7 (C-13), 137.4 (C-4), 140.5 (C-15 or C-3), 140.8 (C-3 or C-15), 144.3 (C-16), 166.3 (C-18), 174.4 (C-17). DCI MS (NH_3) *m/z* (rel. int.): 524 $[M + NH_4]^+$ (2), 506 (0.4), 404 (28), 363 (22), 362 (100), 180 (83).

Enzymatic hydrolysis of compound **16**. To **16** (3 mg) dissolved in 0.5 ml MeOH, 20 mg cellulase (900 CU mg^{-1}, Roth) in 1 ml H_2O were added. After stirring for 12 hr at room temp. 10 ml H_2O were added and the mixt. extracted with $CHCl_3$ (3 × 5 ml). The organic phase was dried and evapd to yield 1.8 mg floribundic acid (**15**). In the aq. phase glucose was identified by TLC [12].

Acid hydrolysis of compound **16**. Compound **16** (2 mg) dissolved in 0.5 ml 2 M HCl–MeOH was kept at 90° for 1 hr. Water was added and the aglycone removed by extraction with $CHCl_3$. Subsequently the aq. phase was neutralized with 2 M NH_3, evapd to dryness and subjected to GC analysis after trifluoroacetylation.

6-*Hydroxycolumbin* (**17**). Amorphous (4 mg). TLC: R_f 0.69 (S-2); anisaldehyde reagent: blue-violet. $[\alpha]_D$ +48° (MeOH; *c* 0.4). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1760, 1740. ^1H NMR (CD_3OD): δ 1.05 (3H, *s*, Me-5), 1.24 (3H, *s*, Me-9), 1.96 (1H, *dd*, J_1 = 15, J_2 = 12.2 Hz, H-11_{ax}), 2.03 (1H, *s*, H-10), 2.33 (1H, *ddd*, J_1 = 15, J_2 = 11.2, J_3 = 8 Hz, H-7_{eq}), 2.36 (1H, *dd*, J_1 = 15, J_2 = 4 Hz, H-11_{eq}), 2.46 (1H, *ddd*, J_1 = 15, J_2 = 10.2, J_3 = 2.2 Hz, H-7_{ax}), 2.74 (1H, *dd*, J_1 = 11.2, J_2 = 2.2 Hz, H-8), 4.20 (1H, *dd*, J_1 = 10.2, J_2 = 8 Hz, H-6), 5.25 (1H, *dd*, J_1 = 5, J_2 = 1.8 Hz, H-1), 5.58 (1H, *dd*, J_1 = 12.2, J_2 = 4 Hz, H-12), 6.22 (1H, *dd*, J_1 = 8, J_2 = 1.8 Hz, H-3), 6.56 (1H, *m*, H-14), 6.58 (1H, *dd*, J_1 = 8, J_2 = 5 Hz, H-2), 7.51 (1H, *m*) and 7.61 (1H, *m*) (H-15 and H-16). ^{13}C NMR (CD_3OD): δ 17.5, 28.5, 28.9, 36.2, 41.0, 42.0, 46.8, 50.9, 71.6, 72.7, 75.2, 84.0, 109.7, 126.5, 132.0, 135.9, 141.5, 145.0, 176.0, 177.2. MS *m/z* (rel. int.): 374.1365 (=$C_{20}H_{22}O_7$) $[M]^+$ (10), 330 (23), 178 (17), 133 (33), 121 (65), 120 (33), 112 (50), 111 (43), 110 (30), 109 (42), 108 (47), 107 (100), 105 (28).

Isomerization of compound **17** *with* NaOH. A soln of **17** (2 mg) in MeOH (1 ml) [5] was heated with 2% aq. NaOH (0.2 ml) at 90° for 5 min. The reaction mixt. was cooled and extracted with CH_2Cl_2 (3 × 3 ml). The organic phase was dried and evapd *in vacuo* to yield 1.6 mg **18**.

6-*Hydroxyisocolumbin* (**18**). Amorphous (4 mg). TLC: R_f 0.66 (S-2); anisaldehyde reagent: blue-violet. $[\alpha]_D$ +30° (MeOH; *c* 0.4). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1760, 1600. ^1H NMR (CD_3OD): δ 1.04 (3H, *s*, Me-9), 1.26 (3H, *s*, Me-5), 1.69 (1H, *ddd*, J_1 = 14, J_2 = J_3 = 8.5 Hz, H-7_{eq}), 1.92 (1H, *dd*, J_1 = 14.5, J_2 = 12.5 Hz, H-11_{ax}), 2.05 (1H, *s*, H-10), 2.30 (1H, *dd*, J_1 = 14.5, J_2 = 4 Hz, H-11_{eq}), 2.53 (1H, *ddd*, J_1 = 14, J_2 = 9.5, J_3 = 8.5 Hz, H-7_{ax}), 3.20 (1H, *dd*, $J_1 \sim J_2 \sim 9$ Hz, H-8), 4.16 (1H, *dd*, $J_1 \sim J_2 \sim 8.5$ Hz, H-6), 5.23 (1H, *dd*, J_1 = 5.5, J_2 = 1.8 Hz, H-1), 5.56 (1H, *dd*, J_1 = 12.5, J_2 = 4 Hz, H-12), 6.22 (1H, *dd*, J_1 = 8, J_2 = 1.8 Hz, H-3), 6.53 (1H, *m*, H-14), 6.57 (1H, *dd*, J_1 = 8, J_2 = 5.5 Hz, H-2), 7.51 (1H, *m*) and 7.59 (1H, *m*) (H-15 and H-16). ^{13}C NMR (CD_3OD): δ 18.9, 26.2, 28.0, 37.4, 42.7, 43.5, 47.3, 60.1, 71.2, 71.4, 75.3, 84.1, 109.7, 125.4, 132.0, 136.4, 141.5, 145.0, 175.8, 177.2. MS *m/z* (rel. int.): 374.1365 ($C_{20}H_{22}O_7$) $[M]^+$ (1), 330 (34), 178 (20), 133 (47), 123 (36), 121 (100), 120 (42), 112 (68), 110 (17), 109 (99), 108 (51), 107 (86), 105 (31).

Isocolumbin (**19**). Crystals from MeOH (8 mg). Mp 175° (lit. [23] mp 185–187° (dec.)). TLC: R_f 0.59 (S-3); anisaldehyde reagent: violet. $[\alpha]_D$ +57° (pyridine, *c* 0.8) (lit. [5] $[\alpha]_D$ +60.1° (pyridine, *c* 1.09)). MS *m/z* (rel. int.): 314 $[M - CO_2]^+$ (7), 247 (13), 246 (18), 231 (17), 153 (100), 122 (23), 121 (29), 112 (48), 108 (57), 107 (77).

Isocolumbin glucoside (**20**). Amorphous (8 mg). TLC: R_f 0.32 (S-2); anisaldehyde reagent: violet. $[\alpha]_D$ +12° (pyridine; *c* 0.45) (lit. [24] $[\alpha]_D$ +27.8° (pyridine, *c* 1.03)). IR, ^1H and ^{13}C NMR in agreement with published data [24].

Penianthic acid methyl ester (**21**). Crystals from Me_2CO (16 mg). Mp 184–186°. TLC: R_f 0.71 (S-1); anisaldehyde reagent: violet. $[\alpha]_D$ −51° ($CHCl_3$; *c* 1.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510, 1730, 1660, 1600. ^1H NMR (pyridine-d_5): δ 1.15 (3H, *s*, Me-9), 1.54 (3H, *s*, Me-5), 1.80 (1H, *dddd*, J_1 = 18.5, J_2 = 11.5, $J_3 \sim J_4 \sim 2$ Hz, H-1_{ax}), 1.93 (1H, *ddd*, J_1 = 18.5, J_2 = 6.5, J_3 = 1.5 Hz, H-6_{eq}), 1.99 (1H, *dd*, J_1 = 14, J_2 = 12 Hz, H-11_{ax}), 2.12 (1H, *dddd*, J_1 = 18.5, $J_2 \sim J_3 \sim 5.5$, $J_4 \sim 1$ Hz, H-1_{eq}), 2.49 (1H, *ddd*, J_1 = 11.5, J_2 = 5.5, $J_3 \sim 1.5$ Hz, H-10), 2.57 (1H, *dd*, J_1 = 18.5, $J_2 \sim 1.9$ Hz, H-6_{ax}), 2.66 (1H, *dd*, J_1 = 14, J_2 = 5.5 Hz, H-11_{eq}), 3.75 (3H, *s*, CO_2Me), 5.46 (1H, *dd*, J_1 = 12, J_2 = 5.5 Hz, H-12), 5.92 (1H, *ddd*, J_1 = 10, J_2 = 5.5, J_3 = 2 Hz, H-2), 6.06 (1H, *dm*, J_1 = 10 Hz, H-3), 6.65 (1H, *m*, H-14), 6.70 (1H, *dd*, J_1 = 6.5, J_2 = 1.9 Hz, H-7), 7.04 (1H, *br s*, OH), 7.66 (1H, *m*) and 7.76 (1H, *m*) (H-15 and H-16). ^{13}C NMR ($CDCl_3$): δ 20.8, 27.1, 29.5, 31.6, 36.7, 40.6, 41.4, 43.9, 52.7 (OMe), 71.1, 78.0, 108.7, 124.5, 125.9, 130.6, 133.4, 135.2, 139.7, 143.7, 169.9, 175.5. EIMS *m/z* (rel. int.) 354 $[M - H_2O]^+$ (100), 201 (15), 159 (15), 157 (34), 151 (48), 133 (35), 131 (18), 128 (99), 119 (20), 109 (43), 108 (15), 107 (75), 105 (36). DCI MS (NH_3) *m/z* (rel. int.): 390 $[M + NH_4]^+$ (100), $[M + H]^+$ 373 (35).

Tinophyllol (**22**). Amorphous (3 mg). TLC: R_f 0.55 (S-1); anisaldehyde reagent: blue-violet. $[\alpha]_D$ −9° (pyridine; *c* 0.33) (lit. [27] $[\alpha]_D$ −19.3° (pyridine; *c* 0.5)). IR, ^1H NMR, ^{13}C NMR and MS in agreement with published data [27].

Tinophylloloside (**23**). Amorphous (11 mg). TLC: R_f 0.49 (S-2); anisaldehyde reagent: violet. $[\alpha]_D$ −16° (MeOH; *c* 1.0) (ref. [28] $[\alpha]_D$ −19.2° (MeOH; *c* 0.25)). IR, ^1H NMR and ^{13}C NMR in agreement with published data [28].

Zenkerin (**24**). Amorphous (15 mg). TLC: R_f 0.46 (S-5); anisaldehyde reagent: red-violet. $[\alpha]_D$ −45° (MeOH; *c* 0.4). IR ν_{max}^{KBr} cm^{-1}: 3400, 1725, 1630. ^1H NMR (CD_3OD): δ 1.03 (3H, *s*, Me-9), 1.29 (3H, *s*, Me-5), 1.2–1.3 (1H, *m*), 1.38–1.50 (1H, *m*), 1.67–1.8 (2H, *m*), 1.84–1.93 (1H, *m*), 1.97 (1H, *dd*, J_1 = 14, J_2 = 11.5 Hz, H-11_{ax}), 2.13 (1H, *dd*, J_1 = 14, J_2 = 6 Hz, H-11_{eq}), 2.16–2.48 (3H, *m*), 2.78 (1H, *dd*, J_1 = 12, $J_2 \sim 3$ Hz, H-8), 2.87 (1H, *dm*, J_1 = 14 Hz, H-6), 3.14–3.67 (8H, *m*, sugar protons), 3.75 (1H, *dd*, J_1 = 11, J_2 = 5 Hz, H-$6'_A$), 3.84 (1H, *dd*, J_1 = 11.5, J_2 = 5.5 Hz, H-$5''_A$), 4.07 (1H, *dd*, J_1 = 11, J_2 = 1.5 Hz, H-$6'_B$), 4.29 (1H, *d*, *J* = 7.5 Hz, H-1''), 5.50 (1H, *dd*, J_1 = 11.5, J_2 = 6 Hz, H-12), 5.54 (1H, *d*, *J* = 8 Hz, H-1'), 6.50 (1H, *m*, H-14), 6.79 (1H, *dd*, $J_1 \sim J_2 \sim 3.5$ Hz, H-3), 7.50 (1H, *m*) and 7.58 (1H, *m*) (H-15 and H-16). ^{13}C NMR (pyridine-d_5): δ 17.4 (C-1, C-2 or C-7), 20.6 (C-7, C-2 or C-1), 22.0 (C-19), 23.7 (C-2, C-7 or C-1), 32.9 (C-20), 35.7 (C-6), 36.5 (C-9), 38.2 (C-5), 45.5 (C-11), 49.1 (C-8), 51.9 (C-10), 67.1 (C-5''), 69.3 (C-6'), 70.2 (C-12), 71.0, 71.1 (C-4', C-4''), 74.0 (C-2'), 74.8 (C-2''),

78.0, 78.1 (C-5', C-3''), 78.7 (C-3'), 95.8 (C-1'), 105.7 (C-1''), 109.6 (C-14), 125.7 (C-13), 137.3 (C-4), 140.5 (C-3 or C-15), 140.8 (C-15 or C-3), 144.3 (C-16), 166.4 (C-18), 174.4 (C-17). DCIMS (NH_3) *m/z* (rel. int.): 656 $[M+NH_4]^+$ (1), 362 (100), 345 (26), 312 (28), 180 (20), 168 (23), 150 (20).

Enzymatic hydrolysis of **24**. To **24** (3 mg) dissolved in 0.5 ml MeOH, 20 mg cellulase in 1 ml H_2O were added. After stirring for 12 hr at room temp. 10 ml H_2O were added and the mixt. extracted with $CHCl_3$ (3 × 5ml). The organic phase was dried and evapd to yield 1.2 mg floribundic acid (**15**). In the aq. phase glucose and xylose were identified among the products by TLC [12].

Acid hydrolysis of compound **24** was performed as described for **16**.

Production of **25** *by acetylation of* **24**. Compound **24** (3 mg) was treated with Ac_2O–pyridine (1:1) for 12 hr at room temp. Purification by CC on silica gel gave amorphous peracetyl zenkerin (**25**) (2.9 mg). TLC: R_f 0.63 (S-4); anisaldehyde reagent: violet. $[\alpha]_D$ −57° ($CHCl_3$; *c* 0.25). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760. ^1H NMR (C_6D_6): δ 0.83 (3H, *s*, Me-9), from δ 0.85 to 1.95 various *m*, 1.18 (3H, *s*, Me-5), 1.57, 1.64, 1.67, 1.76 1.98, 2.07 (3H each, *s*, Ac), 3.05 (1H, *dd*, J_1 = 12, J_2 = 8.5 Hz, H-5''$_B$), 3.08 (1H, *m*), 3.35 (1H, *dd*, J_1 = 11, J_2 = 5 Hz, H-6'$_A$), 3.4 (1H, *ddd*, J_1 = 10, J_2 = 5, J_3 = 1.5 Hz, H-5'), 3.86 (1H, *dd*, J_1 = 11, J_2 = 1.5 Hz, H-6'$_B$), 3.95 (1H, *dd*, J_1 = 12, J_2 = 5 Hz, H-5''$_A$), 4.37 (1H, *d*, J = 6.5 Hz, H-1''), 4.61 (1H, *dd*, J_1 = 11, J_2 = 6 Hz, H-12), 5.03 (1H, *ddd*, J_1 = 8.5, J_2 = 8, J_3 = 5 Hz, H-4''), 5.24 (1H, *dd*, J_1 = 8.5, J_2 = 6.5 Hz, H-2''), 5.29 (1H, *dd*, $J_1 \sim J_2 \sim$ 9.5 Hz, H-4'), 5.39 (1H, *dd*, $J_1 \sim J_2 \sim$ 8 Hz, H-3''), 5.43 (1H, *dd*, $J_1 \sim J_2 \sim$ 9.5 Hz, H-3'), 5.52 (1H, *dd*, J_1 = 9.5, J_2 = 8.5 Hz, H-2'), 5.86 (1H, *d*, J = 8.5 Hz, H-1'), 6.20 (1H, *m*, H-14), 6.54 (1H, *dd*, $J_1 \sim J_2 \sim$ 3.5 Hz, H-3), 7.07 (1H, *m*) and 7.19 (1H, *m*) (H-15 and H-16). DCIMS (NH_3) *m/z* (rel. int.): 908 $[M+NH_4]^+$ (56), 522 (25), 462 (20), 346 (85), 344 (87), 327 (100), 294 (62), 259 (51).

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