Phenols, Acetylenes, and Sesquiterpene Lactones from *Inulanthera nuda*

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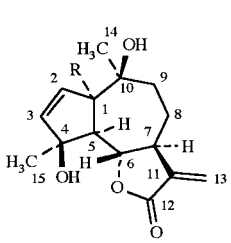
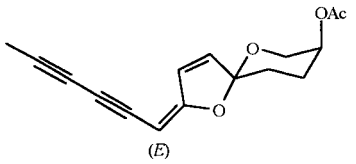
Five new compounds, the sesquiterpene lactones **1** and **2**, the 2*H*-pyran-2-one **9**, the flavone glycoside **10**, and the organic diacid **11** were isolated from the CH_2Cl_2 extract (**1** and **2**) and from the BuOH part of the MeOH extract (**9**–**11**) of the aerial parts of *Inulanthera nuda* KÄLLERSJÖ (Asteraceae). The structures were established by spectroscopic methods, including UV, IR, EI-MS, D/CI-MS and TSP-MS, ^{1}H- and ^{13}C-NMR as well as two-dimensional NMR techniques. The sesquiterpene lactones were identified as 1α,4β,10β-trihydroxyguaia-2,11(13)-dieno-12,6α-lactone (**1**) and 4β,10β-dihydroxy-1α-methoxyguaia-2,11(13)-dieno-12,6α-lactone (**2**), while **9** was found to be 4-(β-D-glucopyranosyloxy)-6-methyl-2*H*-pyran-2-one, **10** was determined as luteolin 7-(2″-*O*-acetyl-β-D-glucuronoside) and **11** as (*Z*)-2-(4-hydroxybenzyl)butenedioic acid. The new compounds were accompanied by six more compounds (**3**–**8**) from the CH_2Cl_2 extract and ten compounds from the BuOH extract (including **12**–**16**). Compounds **3** and **4** were identified as (*E*)- and (*Z*)-*O*-acetyldendranthemenol, while the structures of **5**–**8** were elucidated as the flavones salvigenin, 5-hydroxy-3′,4′,6,7-tetramethoxyflavone, eupatorin, and cirsilineol, respectively, from their spectroscopic data (EI- and D/CI-MS, UV, ^{1}H- and ^{13}C-NMR). Compounds **12**–**16** were determined as scopolin, maltol 3-glucoside, and the flavone glycosides luteolin 7-glucuronide, apigenin 7-glucuronide, and scutellarein 7-glucuronide based on spectral evidences (D/CI- and TSP-MS, UV, ^{1}H- and ^{13}C-NMR). The additional five compounds from the BuOH extract were identified on-line by HPLC-MS as chlorogenic acid, 3,4-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, and 3,4-dicaffeoylisoquinic acid. The identification was based on comparison of retention times and UV and mass spectra with authentic samples.

1. Introduction. – Many species of the Asteraceae family are well-known for their use in traditional and popular medicine. Most of the activities are due to their content of sesquiterpenes, polyacetylenes, or flavonoids [1]. In the course of our search for new antifungal and antibacterial compounds, a phytochemical investigation of *Inulanthera nuda* KÄLLERSJÖ (Asteraceae) was carried out. *I. nuda* is an erect, perennial herb which has been reported to occur only in Eastern and Southern Zimbabwe [2]. The genus *Inulanthera* has recently been established on morphological and chemotaxonomic evidence [3]. It consists of nine former *Athanasia* spp. and one former *Pentzia* sp. Only little phytochemical work has been carried out on this genus [4–7], which prompted us to start the work on this plant.

2. Results and Discussion. – Dried and powdered aerial parts were extracted at room temperature successively with CH_2Cl_2 and MeOH. The MeOH extract was then suspended in H_2O and further extracted with AcOEt and BuOH. Both the CH_2Cl_2 and



1[1] R = OH
2[1] R = CH_3O



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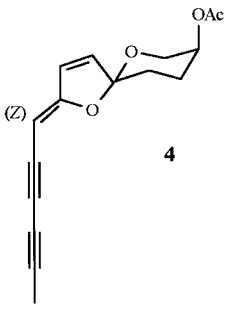

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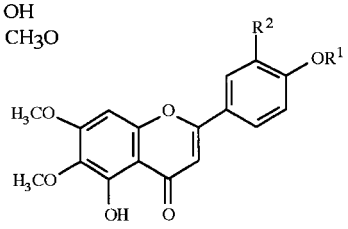
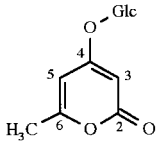


	R^1	R^2
5	CH_3	H
6	CH_3	OCH_3
7	CH_3	OH
8	H	OCH_3



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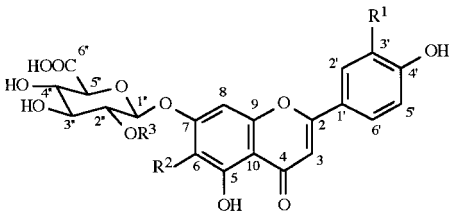
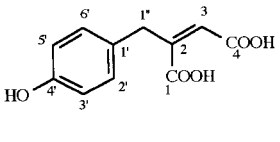


	R^1	R^2	R^3
10	OH	H	Ac
14	OH	H	H
15	H	H	H
16	H	OH	H



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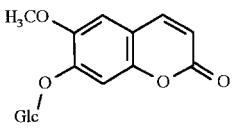
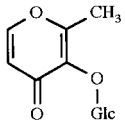


12



13

Glc = β-D-glucopyranosyl

[1]) Trivial numbering; for systematic names, see *Exper. Part.*

the BuOH extracts were fractionated by a combination of open column chromatography (CC), medium-pressure liquid chromatography (MPLC), centrifugal partition chromatography (CPC), and gel filtration on *Sephadex LH-20*. The CH_2Cl_2 extract afforded compounds **1**–**8**, while compounds **9**–**16** were isolated from the BuOH extract.

Compounds **3** and **4** [8] were identified as the spiroacetal enol ethers (*E*)- and (*Z*)-*O*-acetyldendranthemenol. The structures of **5**–**8** [9–12] were elucidated as the flavones salvigenin, 5-hydroxy-3′,4′,6,7′-tetramethoxyflavone, eupatorin, and cirsilineol, respectively, from their spectroscopic data (EI- and D/CI-MS, UV, ^{1}H- and ^{13}C-NMR). Compounds **12**–**16** [13–16] were determined as scopolin, maltol 3-glucoside, and the flavone glycosides luteolin 7-glucuronide, apigenin 7-glucuronide, and scutellarein 7-glucuronide based on spectral evidence (D/CI- and TSP-MS, UV, ^{1}H- and ^{13}C-NMR). The five main constituents of the BuOH extract (besides **9**–**16**) were identified on-line by HPLC-MS as chlorogenic acid, 3,4-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, and 3,4-dicaffeoylisoquinic acid; their identification was based on comparison of retention times and UV and mass spectra with those of authentic samples.

Compounds **1**, **2**, and **9**–**11** proved to be new natural products. The structure of **1** was established by D/CI-MS, ^{13}C- and ^{1}H-NMR spectra and by ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, and NOE experiments which suggested that **1** was a sesquiterpene lactone of the guaianolide type. The relative configuration of a 1α,4β,10β-trihydroxyguaia-2,11(13)-dieno-12,6α-lactone was confirmed by an X-ray crystal-structure analysis of **1** (*Fig. 1*). The absolute configuration of **1** was not determined. A compound with a different configuration at C(4) and C(10) has been isolated from *Artemisia rutifolia* (Asteraceae) [17].

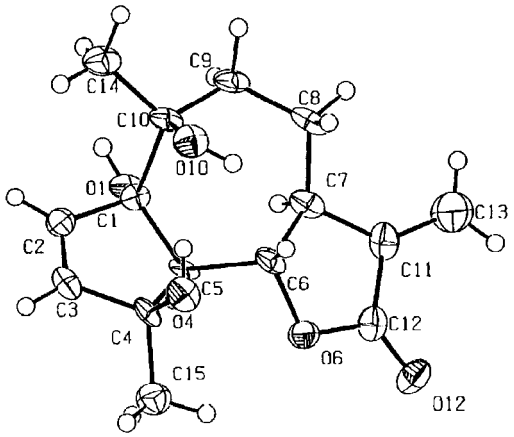


Fig. 1. *View of* **1** *drawn with the program Xtal_GX*

The molecular mass of **1** was obtained by the D/CI-MS which showed the molecular ion M^+ at *m*/*z* 280. In the ^{13}C-NMR and DEPT spectra, 15 C-atoms were found. Of these, five were quaternary, three CH_2 groups, five CH groups, and two Me groups. This allowed to establish the molecular formula as $C_{15}H_{20}O_5$, suggesting that the compound was a sesquiterpene.

The ^{1}H-NMR spectrum showed the presence of the two methylene protons of an α-methylene-γ-lactone moiety at δ 6.05 (d, J = 3.4 Hz) and 5.65 (d, J = 3.0 Hz). The signals at δ 5.87 (d, J = 5.7 Hz) and 5.70 (d, J =

5.7 Hz) were attributed to two olefinic protons at a disubstituted double bond. Another low-field signal was found at δ 4.58 (t, $J = 9.7$ Hz, 1 H). The low-field shift of this t was caused by the proximity of an O-atom, as observed with H–C(6) in sesquiterpene lactones. In the COSY spectrum, a correlation of H–C(6) was observed with a proton at δ 3.09 (m) and another proton at δ 2.15 (d, $J = 9.7$ Hz), which were identified as H–C(7) and H–C(5), respectively, by comparison with the ^{1}H-NMR data of reference sesquiterpene lactones [18]. Furthermore, the signals for two Me groups at δ 1.33 (s, Me(15)) and 1.16 (s, Me(14)) and three OH protons at δ 5.47 (s, OH–C(10)), 5.27 (s, OH–C(1)), and 4.20 (s, OH–C(4)) were observed. These data suggested that **1** was a sesquiterpene lactone of the guaianolide type. The ^{13}C-NMR and the DEPT spectra established that all O-substituents were attached to quaternary C-atoms (δ 96.4, 78.3, and 74.9). The positions of the OH groups were finally determined by two-dimensional NMR techniques (^{1}H,^{13}C-HSQC and ^{1}H,^{13}C-HMBC experiments). Thus, the O-substituents had to be in positions C(1), C(4), and C(10).

The relative configurations at the chiral centers C(1), C(4), C(5), C(6), and C(7) were established by the ^{1}H-NMR data and the NOE experiments. The *trans* configuration of H–C(6) relative to H–C(5) and H–C(7) was deduced from the coupling constants $J(5,6) = 9.7$ and $J(6,7) = 9.7$ Hz [19]. This was confirmed by the coupling constants $J(7,13a) = 3.0$ Hz and $J(7,13b) = 3.4$ Hz. According to *Samek* [20], the following rule can be applied for natural sesquiterpene lactones: $|^4J|$ (*trans*-lactone) $\geq 3 \geq |^4J|$ (*cis*-lactone). The relative configuration of the OH groups was checked by NOE experiments. The following NOEs were observed: H–C(6)/OH–C(10) and OH–C(4), Me(14)/H–C(2) and OH–C(10), Me(15)/H–C(3) and OH–C(4), and H–C(5)/OH–C(1) and H–C(7).

The comparison of the spectral data of **2** with those of **1** and an X-ray crystal-structure analysis (*Fig. 2*) established the structure of **2** with the relative configuration of a 4β,10β-dihydroxy-1α-methoxyguaia-2,11(13)-dieno-12,6α-lactone.

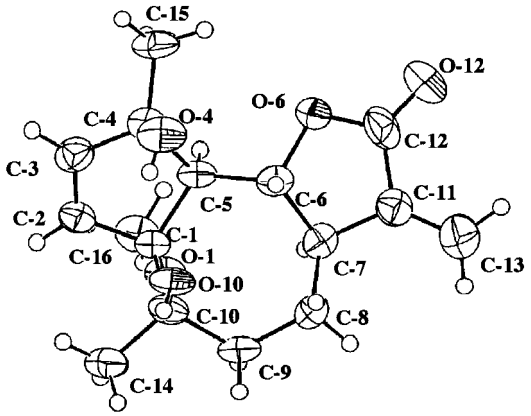


Fig. 2. *View of* **2** *drawn with the program Xtal_GX*

The D/CI-MS of **2** showed a molecular ion M^+ at m/z 294, indicating a difference of 14 amu from the mass of **1**. Thus, the molecular formula was deduced as $C_{16}H_{22}O_5$. The ^{1}H-NMR spectrum of **2** was very similar to that of **1**. However, the signal of a MeO group (δ 31.0, s) but of only two OH groups (δ 5.60 and 4.16, both s) were found indicating that **2** was a methyl derivative of **1**. The position of the MeO group was determined by an NOE experiment. Irradiation at the MeO signal gave an enhancement of the d of H–C(2) (δ 5.77) and the d of H–C(5) (δ 2.29). The relative configurations at C(1) and C(4) were deduced from NOE experiments. The NOEs Me(15)/H–C(5) and MeO/H–C(5) gave evidence of the α-orientation of the Me and MeO substituents. The α-orientation of Me–C(10) was confirmed by the X-ray analysis (*Fig. 2*).

Compound **9** exhibited only one maximum in the UV spectrum at λ 285 nm. Acid hydrolysis allowed to obtain the sugar moiety. It was identified by TLC as D-glucose by comparison with an authentic sample. The molecular formula of **9** was established by

D/CI-MS as $C_{12}H_{16}O_8$. The ^{1}H- and ^{13}C-NMR data were only compatible with the presence of a 2*H*-pyran-2-one moiety which was confirmed by the IR spectrum $\tilde{\nu}$(C=O) at 1710 and 1700 cm^{-1}; *cf.* the literature values for 2*H*-pyran-2-ones (1680–1730 cm^{-1}) [21] and 4*H*-pyran-4-ones (1590–1630 cm^{-1}) [22]. The structure of **9** was finally established as 4-(β-D-glucopyranosyloxy)-6-methyl-2*H*-pyran-2-one.

A molecular mass of 288 amu was deduced from D/CI-MS of **9**, giving a peak of the ammonium adduct $[M+NH_4]^+$ at *m*/*z* 306. The main fragment at *m*/*z* 144 $[(M+NH_4)-162]^+$ indicated the loss of a hexosyl unit.

In the ^{1}H-NMR spectrum, two ^4J-related protons were found at δ 6.10 (*d*, *J* = 1.8 Hz) and 5.62 (*d*, *J* = 1.8 Hz). The remaining signals were attributed to the sugar protons (δ 5.02 (*d*, *J* = 7.4 Hz, H–C(1′)); 3.68 (*d*, *J* = 12.3 Hz, H_b–C(6′)); 3.47 (*dd*, *J* = 12.3, 5.9 Hz, H_a–C(6′)); 3.1–3.4 (4 H, signal pattern unclear due to overlapping)) and to a Me group (δ 2.20, *s*). The value of the coupling constant of the anomeric-proton signal (*J* = 7.4 Hz) established a β-D-glucopyranoside [23]. The positions of the substituents at the 2*H*-pyran-2-one moiety were determined by means of the ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra. As all of the three low-field C-signals (δ 169.1, 163.8, 163.1) arose from quaternary C-atoms, the position C(6) had to be substituted with the Me group. Substitution at C(6) by the glucosyl moiety would not be compatible with the aforementioned data.

Compound **10** exhibited bands (λ_{max} 207, 253, 264, and 346 nm) characteristic for a flavone in the UV spectrum [24], and the TSP-MS suggested the structure of a tetrahydroxy-substituted flavone. By means of the ^{13}C-NMR spectrum, the aglycone was identified as luteolin. The remaining signals agreed with an acetylated glucuronic or galacturonic acid which fitted well with the loss of 218 amu in the TSP-MS spectrum. The nature of the uronic acid was determined by the ^{1}H-NMR and ROESY data, and the glycosylation site by the UV spectrum after addition of shift reagents [24]. Compound **10** was finally subjected to basic hydrolysis to remove the acetyl group. The hydrolysis product was identified as luteolin 7-(β-D-glucuronoside) (**14**) by TLC comparison (AcOEt/MeOH/H_2O/AcOH 13 : 3 : 3 : 4) with an authentic sample (isolated from *Inulanthera nuda*). Thus, **10** was determined as luteolin 7-(2″-*O*-acetyl-β-D-glucuronoside).

In the TSP-MS of **10**, a pseudo-molecular ion $[M+H]^+$ was observed at *m*/*z* 505, and the protonated aglycone at *m*/*z* 287 (100%). The presence of an acetyl group was confirmed by the signal of Me group at δ 1.93 (*s*) in the ^{1}H-NMR spectrum. The site of acetylation was determined by means of ^{1}H-NMR spectroscopy. The protons of the sugar moiety gave signals at δ 5.35 (*d*, *J* = 8.1 Hz, H–C(1″)), 4.77 (*dd*, *J* = 8.8, 8.1 Hz, H–C(2″)), 3.71 (*d*, *J* = 9.4 Hz, H–C(5″)), 3.59 (*t*, *J* = 8.8 Hz, H–C(3″)), and 3.49 (*dd*, *J* = 9.4, 8.8 Hz, H–C(4″)) which were attributed with a COSY experiment. It is well-known that acetylation is causing a low-field shift of the proton which is located at the same C-atom as the AcO group [25]. The low-field shift of H–C(2″) ($\Delta\delta$ = 1.52, compared to **15**), therefore, established acetylation at OH–C(2″).

In the UV spectrum of **10** the shift of band I (+ 45 nm) on addition of NaOMe suggested the presence of a free OH group at C(4′). A bathochromic shift (+ 24 nm) of band I on addition of $NaOAc/H_3BO_3$ indicated the formation of a complex of boric acid with OH–C(3′) and OH–C(4′). The shift (+ 39 nm) on addition of $AlCl_3$/HCl finally evidenced a free OH group at C(5). Thus, the substituents at C(5), C(3′), and C(4′) were free OH groups, and the sugar moiety had to be attached at C(7).

The values *J*(3″,4″) = 8.8 (*trans*-diaxial) and *J*(4″,5″) = 9.4 Hz in the ^{1}H-NMR indicated unambiguously only the presence of glucuronic acid. This was confirmed by a ROESY experiment, where an *Overhauser* effect was observed between H–C(2″) and H–C(4″). In glucuronic acid, these protons are both axially positioned, while in galacturonic acid, H–C(4″) is in an equatorial position.

Compound **11**, finally, showed two absorption bands in the UV spectrum at λ 220 and 274 nm, the latter band being not very intense (log ε 3.27). From this UV spectrum, the presence of an aromatic ring without a further conjugated double bond was

deduced. The D/CI-MS (molecular formula $C_{11}O_{10}O_5$), NMR spectra, and an INEPT experiment established the structure proposed for **11**.

In the D/CI-MS of **11**, a molecular ion M^+ was observed at *m/z* 222, which was confirmed by the peak of the ammonium adduct $[M+NH_4]^+$ at *m/z* 240. The ^{1}H-NMR spectrum showed signals for an aromatic OH group at δ 9.18 (br. *s*) and *AA′BB′* system similar to those found, *e.g.*, in **15** and **16**, with signals at δ 6.97 (*dd*, $J=8.6$, 1.9 Hz, 2 H) and 7.36 (*dd*, $J=8.6$, 1.9 Hz, 2 H). A signal at δ 5.85 (*t*, $J=1.2$ Hz, 1 H), which was attributed to the olefinic proton, and the *d* of a methylene group at δ 3.44 (*d*, $J=1.2$ Hz, 2 H) were also present. Comparison with the ^{1}H-NMR spectra of **15** and **16** suggested the presence of a *para*-hydroxybenzyl moiety in **11**. This was confirmed in the ^{13}C-NMR spectrum by the signals at δ 155.4, 129.9, 129.7, and 114.9, the resonances at δ 129.9 and 114.9 actually arising each from two C-atoms due to the symmetry of the *para*-hydroxyphenyl moiety. The remaining signals in the ^{13}C-NMR spectrum were attributed to a butenedioic-acid moiety (δ 167.3, 167.2, 147.7, and 131.9) and a CH_2 signal at δ 40.3, which was supposed to link the aromatic moiety with the acid unit. Finally, a selective INEPT experiment, irradiating the ^{1}H-NMR signal of the CH_2 established that the CH_2 group was linking the acryl moiety with the acid unit. The configuration at C(2) of **11** was calculated by means of the shift of H–C(3) in the ^{1}H-NMR spectrum. According to *Pretsch et al.* [21], H–C(3) would appear at δ 6.71 for the (*E*)-configurated and at δ 6.08 for the (*Z*)-configurated isomer. In fact, H–C(3) was observed at δ 5.85, indicating (*Z*)-configuration. This was supported by comparison with the chemical shift of the olefinic protons (90 MHz, $CDCl_3$) of dimethyl (*E*)- and (*Z*)-veratrylidensuccinate (= dimethyl (*E*)- and (*Z*)-2-[(3,4-dimethoxyphenyl)methylene]butanedioate) [26]: the (*E*)-isomer gave rise to a *s* at δ 7.83, while the (*Z*)-isomer showed a *t* at δ 6.76 (allylic coupling with the CH_2 group). As in the ^{1}H-NMR spectrum of **11** also a *t* with a small *J* value was observed, the compound had to be (*Z*)-configurated.

The compounds which were isolated in sufficient amount from *I. nuda* were submitted to a screening against *Candida albicans*, *Cladosporium cucumerinum*, *Bacillus subtilis*, and *Escherichia coli* on TLC [27]. While the sesquiterpene lactones and the flavonoids isolated from the CH_2Cl_2 extract were devoid of any growth-inhibiting activity towards these microorganisms, the spiroacetal enol ether **3** inhibited growth of *C. cucumerinum* and *E. coli* on TLC. The activities against *C. cucumerinum* and *E. coli* of **3** were also determined in an agar dilution assay [28]. The compounds isolated from the BuOH extract generally did not inhibit growth of any of the tested microorganisms. An exception was compound **12** which showed an activity against *C. cucumerinum* on TLC. None of the tested compounds exhibited any activity against *B. subtilis*. The results of these tests are listed in the *Table*.

Table. *Antifungal and Antibacterial Activities of Compounds Isolated from* I. nuda

	Cladosporium cucumerinum		*Candida albicans*	*Escherichia coli*	
3	5[a])	80[b])	[d])	4[a])	>40[b])
12	10[a])	n.d.[c])	[d])	[d])	n.d.[c])
Propiconazole	0.1[a])				
Amphotericin B	1[a])	10[b])	1[b])		
Chloramphenicol				0.1[a])	10[b])

[a]) Minimal amount [μg] of compound to inhibit growth on a silica gel TLC plate. [b]) Minimal inhibition concentration *MIC* [μg/ml] of compound in an agar-dilution assay. [c]) *MIC* not determined. [d]) No activity observed.

The isolated molecules agree well with the type of molecules isolated before from *Inulanthera* spp. The occurrence of spiroacetal enol ethers as well as the presence of sesquiterpenes of the guaianolactone- or eudesmanolacton-type seems to be common

to all members of the genus. The lack of furanosesquiterpenes in *I. nuda* further justifies the revision of the South African genera belonging to the tribe Anthemidae, carried out by *Källersjö* in 1985 [3] who reported the formation of the new genus *Inulanthera*, which was composed of former *Athanasia* spp. as well as one former *Pentzia* sp. One characteristic of the new genus was the presence of acetylenic compounds and the lack of furanosesquiterpenes.

Two of the new compounds from the BuOH extract, 4-(β-D-glucopyranosyloxy)-6-methyl-2*H*-pyran-2-one (**9**) and (*Z*)-2-(4-hydroxybenzyl)butenedioic acid (**11**), are rather unusual plant metabolites in the Asteraceae family. While the occurrence of 2*H*-pyran-2-one ($=\alpha$-pyrone) derivatives is known in the tribe Anthemidae [29], it has not been reported to occur in the genus *Inulanthera*. The butenedioic acid is of interest as it is the first time that an aromatic moiety substituted with a C_5 unit is reported in the genus.

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Experimental Part

General. TLC: Silica gel *60 F_{254}* Al sheets (*Merck*). Open column chromatography (CC): *Sephadex LH-20* (*Pharmacia*) and silica gel (40–63 μm, *Merck*). Centrifugal partition chromatography (CPC): *CCC-1000* (*Pharma-Tech Research Corp.*). Medium-pressure liquid chromatography (MPLC): home-packed silica gel column (15–40 μm, 460 × 70 mm i.d., *Merck*) and *LiChroprep RP-18* columns (15–25 μm, 460 × 15 mm i.d.; 25–40 μm, 460 × 36 mm i.d., *Merck*). Anal. HPLC: *Hewlett-Packard-1050* instrument equipped with a photodiode array detector; *Nova-Pak RP-18* (4 μm; 150 × 3.9 mm i.d.); MeCN/H_2O 15 : 85 →100 : 0 in 30 min, 0.05% CF_3COOH, 1 ml/min for compounds of the CH_2Cl_2 extract; MeCN/H_2O 10:90 →100 : 0 in 50 min, 0.05% CF_3COOH, 1 ml/min for compounds of the MeOH extract. M.p.: *Mettler-FP-80/82* hot-stage apparatus; uncorrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. UV = λ_{max} (log ε) in m. IR: *Perkin-Elmer-781* spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR: *Varian-Unity-Innova-500* and *Varian-VXR-200* spectrometers; δ in ppm rel to $SiMe_4$; *J* in Hz; C-multiplicities from DEPT experiments. EI-MS, D/CI-MS, and TSP-MS: *Finnigan-MAT-TSQ-700* triple-stage quadrupole instrument; *m/z* (rel. int. in %).

Plant Material. The aerial parts of *Inulanthera nuda* were collected in 1992 in the Nyanga National Park, eastern highlands, Zimbabwe. A voucher specimen is deposited at the Institut de Pharmacognosie et Phytochimie in Lausanne (voucher No. 92102).

Extraction and Isolation. The powdered aerial parts (440 g) were extracted at r.t. successively with 3 × 5 l of CH_2Cl_2 and 3 × 5 l of MeOH to afford 60 and 30 g, respectively, of extract. A portion (25.4 g) of the CH_2Cl_2 extract was fractionated by MPLC (silica gel) to give 11 fractions (*I–XI*). *Fr. III* was further separated by MPLC (*RP-18*, MeOH/H_2O 3 : 2) to yield 13 fractions (*1–13*). Compound **3** (54.4 mg) was obtained from *Fr. 6* as colorless plates after gel filtration (*Sephadex LH-20*, $CHCl_3$/MeOH 1 : 1). *Fr. IV* was also subjected to MPLC (MeOH/H_2O 2 : 3). A further separation step by CC (silica gel) yielded **4** (4.7 mg) as dark brown oil. Compounds **2**, **5**, and **6** were obtained after subjecting *Fr. V* to MPLC (MeOH/H_2O 2 : 3). While **2** (28.0 mg) and **5** (144.4 mg) crystallized as colorless or yellow plates, respectively, from $CHCl_3$/MeOH, **6** (61.3 mg) was obtained as amorphous yellow powder. *Fr. VII* was also subjected to MPLC (MeOH/H_2O 2 : 3) which yielded **1** (432.9 mg). This compound crystallized already in the tubes of the fraction collector from the elution solvent. MPLC (MeOH/H_2O 3 : 2) of *Fr. X* finally yielded **7** and **8** (3.4 mg each). Both compounds crystallized from $CHCl_3$/MeOH as yellow needles. Their NMR analysis revealed that **7** and **8** are two isomers.

A portion (20.2 g) of the MeOH extract was suspended in H_2O and extracted successively with AcOEt and BuOH to yield 4.4 and 5.4 g of extract, respectively. From the BuOH extract, 5.1 g were subjected to CPC (lower phase of $CHCl_3$/MeOH/H_2O/i-PrOH 7:6:1:4). Thus, 13 fractions were obtained (*I–XIII*). During the evaporation process of *Fr. VII*, a white powder precipitated. It was washed with $CHCl_3$, yielding finally 5.2 mg of pure **12**. *Fr. IX* was subjected to MPLC (*RP-18*, MeOH/H_2O 1:9). With this technique, **9** (144.3 mg) and **13** (11.5 mg) were obtained as colorless amorphous powders. *Fr. XII* was further separated by gel filtration (*Sephadex LH-20*) to yield *Fr. 1–7*. From *Fr. 6*, **14** (13.3 mg) precipitated as amorphous yellow powder. *Fr. 5* was subjected to MPLC (*RP-18*, MeOH/H_2O 1:9) to give **15** (6.3 mg) as yellow amorphous powder and, after a

purification step on anal. HPLC (MeCN/H_2O 7:43), 1.5 mg of **16**, again as yellow amorphous powder. Finally, *Fr. XIII* was separated by MPLC (*RP-18*, MeOH/H_2O 1:4): **10** (6.6 mg) and **11** (18.7 mg) as yellow or colorless amorphous powder, respectively. The five main compounds in the BuOH extract were identified on-line by HPLC/TSP-MS (*Nova-Pak-RP-18* (4 μm, 150 × 3.9 mm i.d.), MeCN/H_2O (+0.05% CF_3COOH) 10:90 → 100:0 in 50 min, flow rate 1 ml/min). The identification (see *General Part*) was based on comparison of retention times, and UV and mass spectra with authentic samples.

*(3*aS*,6*R*,6a*R*,9*R*,9a*R*,9b*R*)-3a,4,5,6,6a,9,9a,9b-Octahydro-6,6a,9-trihydroxy-6,9-dimethyl-3-methyleneazuleno[4,5-*b*]furan-2(3*H*)-one* (**1**). Colorless plates. M.p. 218–221°. $[\alpha]_D = +134$ ($CHCl_3$, $c = 0.60$). IR: 3350, 2920, 1725, 1425, 1310, 1255, 1115, 1010, 975, 960, 930, 820, 785. ^{1}H-NMR (200 MHz, (D_6)DMSO)[1]): 6.05 (d, $J = 3.4$, H_b–C(13)); 5.87 (d, $J = 5.7$, H–C(3)); 5.70 (d, $J = 5.7$, H–C(2)); 5.65 (d, $J = 3.0$, H_a–C(13)); 5.47 (s, OH–C(10)); 5.27 (s, OH–C(1)); 4.58 (t, $J = 9.7$, H–C(6)); 4.20 (s, OH–C(4)); 3.09 (m, H–C(7)); 2.28 (m, H_a–C(9)); 2.15 (d, $J = 9.7$, H–C(5)); 1.99 (m, H_a–C(8)); 1.57 (m, H_b–C(8)); 1.43 (m, H_b–C(9)); 1.33 (s, Me(15)); 1.16 (s, Me(14)). ^{13}C-NMR (50 MHz, (D_6)DMSO)[1]): 170.0 (C(12)); 140.0 (C(11)); 139.1 (C(3)); 136.8 (C(2)); 119.6 (C(13)); 88.0 (C(1)); 82.4 (C(6)); 78.6 (C(4)); 74.8 (C(10)); 63.8 (C(5)); 41.4 (C(7)); 35.3 (C(9)); 28.3 (C(14)); 26.0 (C(15)); 25.1 (C(8)). EI-MS: 216(32), 204(38), 202(21), 201(43), 192(20), 187(22), 186(82), 175(22), 174(100), 173(28), 167(20). D/CI-MS: 299(19), 298 (100, $[M + NH_4]^+$), 280 (76, M^+, $C_{15}H_{20}O_5^+$), 263(6).

*(3*aS*,6*R*,6a*R*,9*R*,9a*R*,9b*R*)-3a,4,5,6,6a,9,9a,9b-Octahydro-6,9-dihydroxy-6a-methoxy-6,9-dimethyl-3-methyleneazuleno[4,5-*b*]furan-2(3*H*)-one* (**2**). Colorless plates. M.p. 182–185°. ^{1}H-NMR (200 MHz, (D_6)DMSO)[1]): 6.04 (d, $J = 3.5$, H_b–C(13)); 6.04 (d, $J = 5.9$, H–C(3)); 5.77 (d, $J = 5.9$, H–C(2)); 5.64 (d, $J = 3.1$, H_a–C(13)); 5.60 (s, OH–C(10)); 4.62 (t, $J = 9.5$, H–C(6)); 4.16 (s, OH–C(4)); 3.10 (s, MeO–C(1)); 2.88 (m, H–C(7)); 2.29 (d, $J = 9.5$, H–C(5)); 2.16 (dd, $J = 13.0$, 7.8, H_a–C(9)); 1.97 (m, H_a–C(8)); 1.52 (m, H_b–C(8)); 1.45 (m, H_b–C(9)); 1.34 (s, Me(15)); 1.11 (s, Me(14)). ^{13}C-NMR (50 MHz, (D_6)DMS)[1]): 169.9 (C(12)); 142.3 (C(3)); 139.9 (C(11)); 133.2 (C(2)); 119.6 (C(13)); 94.6 (C(1)); 82.4 (C(6)); 78.3 (C(4)); 74.9 (C(10)); 53.9 (C(5)); 50.0 (*Me*O–C(1)); 41.1 (C(7)); 35.3 (C(9)); 28.4 (C(14)); (25.8 (C(15)); 25.1 (C(8)). EI-MS: 276 (100, $[M - NH_4]^+$), 258(18), 247(11), 245(10), 231(11), 167((5), 125(11), 110(22), 109(12). D/CI-MS: 294 (10, M^+), 208(19), 192(100), 180(31), 104(16).

Crystallographic Data **1**. $C_{15}H_{20}O_5$. Colorless plates, 0.49 × 0.38 × 0.17 mm; orthorhombic, space group $P2_12_12_1$; $a = 5.924(1)$, $b = 12.831(3)$, $c = 18.858(5)$ Å, $Z = 4$; $T = 213$ K, 2968 reflections measured, 2516 independent reflections (R_{int} 0.032), 2433 observed reflections ($I > 2\sigma(I)$); final R_1 0.0775, R_{w2} 0.162 (observed data); goodness of fit 1.326, residual density max/min 0.284/−0.265 e·Å^{-3}; absorption coefficient $\mu = 0.097$ mm^{-1}; no correction for absorption was applied.

2: $C_{16}H_{22}O_5$. Colorless plates, 0.76 × 0.46 × 0.15 mm; monoclinic, space group $P2_1$; $a = 9.7997(10)$, $b = 9.4623(10)$, $c = 16.483(2)$ Å, $\beta = 94.06(1)°$, $Z = 4$; $T = 293$ K, 5594 reflections measured, 2861 independent reflections (R_{int} 0.032), 2624 observed reflections ($I > 2\sigma(I)$), final R_1 0.0751, R_{w2} 0.1244 (observed data); goodness of fit 1.353, residual density max/min 0.213/−0.190 e·Å^{-3}; absorption coefficient $\mu = 0.095$ mm^{-1}; no correction for absorption was applied.

Intensity data were collected at r.t. for **2** and at −60° for **1** using a *Stoe-AED2-4-circle* diffractometer MoK_α graphite monochromated radiation (λ 0.71073 Å) with $\omega/2\theta$ scans in the 2θ range 5–51°. The hydroxy H-atoms were located from difference maps and allowed to refine isotropically. The remainder of the H-atoms were included in calculated positions and allowed to ride on the corresponding C-atom. The non-H-atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. The structures were solved by direct methods using the programme SHELXS-86 [30]. The refinement and all further calculations were carried out using SHELXL-93 [31]. The molecular structures were drawn with the program Xtal_GX [32].

The bond lengths and angles are normal within experimental error. No attempt was made to determine the absolute configuration of the molecules. In both **1** and **2** there exists an intra-molecular H-bond linking OH groups O4 (donor) and O10 (acceptor) (see *Figs. 1* and *2*). In the crystal of **1**, the molecules are linked by two inter-molecular H-bonds to form a two-dimensional sheet extending in the *ab* plane. The first involves the carbonyl O12 (acceptor) and the OH group O1 (donor), the second involves the OH groups O1 (acceptor) and O10 (donor). In **2**, there are two independent molecules per asymmetric unit which do not differ from one another in any significant manner. In the crystal of **2**, the individual independent molecules are linked to form chains *via* an intermolecular H-bond involving OH groups O4 (donor) and O10 (acceptor).

*4(β-*D*-Glucopyranosyloxy)-6-methyl-2*H*-pyran-2-one* (**9**). Colorless amorphous powder. M.p. >185° (dec.). $[\alpha]_D = -48$ (H_2O, $c = 0.61$). IR: 1710, 1700, 1630, 1550, 1440, 1240, 1180, 820. UV (MeOH): 203(3.88), 285(3.30). ^{1}H-NMR (200 MHz, (D_6)DMSO): 6.10 (d, $J = 1.8$, H–C(3)); 5.62 (d, $J = 1.8$, H–C(5)); 5.02 (d, $J = 7.4$, H–C(1′)); 2.20 (s, Me–C(6)). ^{13}C-NMR (50 MHz, (D_6)DMSO): 169.1 (C(4)); 163.8 (C(2));

163.1 (C(6)); 99.8 (C(5)); 99.1 (C(1′)); 90.0 (C(3)); 77.3 (C(3′)); 76.3 (C(5′)); 72.9 (C(2′)); 69.4 (C(4′)); 60.5 (C(6′)); 19.4 (*Me*–C(6)). D/CI-MS: 306 (53, $[M+NH_4]^+$), 197(15), 161(50), 144(100).

*2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-oxo-4*H*-1-benzopyran-7-yl 2-*O*-Acetyl-β-*D*-glucopyranosiduronic Acid* (**10**). Yellow amorphous powder. M.p. >300°. UV (MeOH): 207(4.38), 253(4.01), 264(3.98), 346(4.06). UV (MeOH + NaOMe): 265, 391. UV (MeOH + NaOAc): 258, 352, 406 (sh). UV (MeOH + NaOAc/H_3BO_3): 258, 370. UV (MeOH + $AlCl_3$): 272, 296, 331, 429. UV (MeOH + $AlCl_3$/HCl): 268, 296, 352, 385. ^{1}H-NMR (500 MHz, (D_6)DMSO): 12.97 (*s*, OH–C(5)); 7.42 (*dd*, *J* = 7.8, 2.5, H–C(6′)); 7.41 (*dd*, *J* = 2.5, H–C(2′)); 6.88 (*d*, *J* = 7.8, H–C(5′)); 6.72 (*d*, *J* = 2.0, H–C(8)); 6.72 (*s*, H–C(3)); 6.36 (*d*, *J* = 2.0, H–C(6)); 5.35 (*d*, *J* = 8.1, H–C(1″)); 4.77 (*dd*, *J* = 8.8, 8.1, H–C(2″)); 3.71 (*d*, *J* = 9.4, H–C(5″)); 3.59 (*t*, *J* = 8.8, H–C(3″)); 3.49 (*dd*, *J* = 9.4, 8.8, H–C(4′)); 1.93 (*s*, MeCOO). ^{13}C-NMR (50 MHz, (D_6)DMSO): 181.9 (C(4)); 170.4 (C(6″)); 169.3 (Me*C*OO–C(2″)); 164.5 (C(2)); 162.0 (C(7)); 161.2 (C(5)); 156.9 (C(9)); 150.0 (C(4′)); 145.8 (C(3′)); 121.2 (C(1′)); 119.2 (C(6′)); 116.0 (C(5′)); 113.5 (C(2′)); 105.6 (C(10)); 103.1 (C(3)); 99.5 (C(6)); 96.8 (C(1″)); 94.7 (C(8)); 74.6 (C(5″)); 73.4 (C(4″)); 72.9 (C(2″)); 71.7 (C(3″)); 20.8 (Me). TSP-MS: 527 (16, $[M+Na]^+$), 505 (72, $[M+H]^+$, $C_{23}H_{21}O_{13}$), 447(27), 302(18), 287 (100, $[M+H-glucuronyl]^+$), 236(25).

2-[(4-Hydroxyphenyl)methyl]but-2-enedioic Acid (**11**). Colorless amorphous powder. M.p. 126–131°. UV (MeOH): 220(4.06), 274(3.27). ^{1}H-NMR (200 MHz, (D_6)DMSO): 9.18 (br. *s*, OH–C(4′)); 6.97 (*dd*, *J* = 8.6, 1.9, H–C(2′), H–C(6′)); 7.36 (*dd J* = 8.6, 1.9, H–C(3′), H–C(5′)); 5.85 (*t*, *J* = 1.2, H–C(3)); 3.44 (*d*, *J* = 1.2, 2 H–C(1″)). ^{13}C-NMR (50 MHz, (D_6)DMSO): 167.3 (C(1) or C(4)); 167.2 (C(4) or C(1)); 155.5 (C(4′)); 147.7 (C(2)); 131.9 (C(3)); 129.9 (C(2′), (C(6′)); 129.7 (C(1′)); 114.9 (C(3′), C(5′)); 40.3 (C(7′)). D/CI-MS: 230 (10, $[M+NH_4]^+$), 239(11), 222 (24, M^+, $C_{11}H_{10}O_5^+$), 119(37), 102(100).

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