Thrombin Inhibitory Constituents from *Duranta repens*

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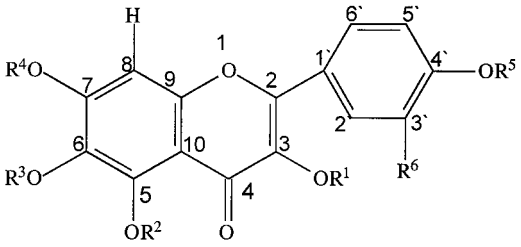
The *C*-alkylated flavonoids 3,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5,6-dimethoxyflavone (**1**), 3,7-dihydroxy-3′-(4-hydroxy-3-methylbutyl)-5,6,4′-trimethoxyflavone (**2**) and the *trans*-clerodane diterpenoids 6β-hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic acid (**3**) and 2β-hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic acid (**4**) were isolated from *Duranta repens*. Their structures and the relative configuration of **3** and **4** were determined by spectroscopic methods (^{1}H- and ^{13}C-NMR, IR, and MS) and 2D-NMR experiments. The known flavonoid **5** is also reported for the first time from this species. The compounds **1**, **3**, and **5** showed significant enzyme-inhibitory activity against thrombin.

1. Introduction. – The genus *Duranta* (Verbenaceae) comprises about 35 species which are evergreen shrubs distributed in tropical and sub-tropical regions. A literature survey revealed that very little phytochemical work has been carried out on the genus *Duranta*, and only some steroids [1], triterpenes [2], and iridoids [3] have so far been reported. *Duranta repens* LINN. is widely distributed in northern parts of Pakistan and finds various medicinal uses in the indigenous system of medicine. The fruits of this plant afford a medicine for the treatment of malaria [4]. The MeOH extract also shows insecticidal and antifeedant properties against *Aedes aegypti* and *Attagenus piceus*, respectively [5]. Our preliminary pharmacological screening of the EtOH extract revealed enzyme-inhibitory activity. This prompted us to carry out the phytochemical studies on this plant.

Herein we report the isolation and structure elucidation of the two new *C*-alkylated flavonoids **1** and **2** and of the new clerodane-type diterpenoids **3** and **4**, along with the known flavonoid **5** reported for the first time from this species [6]. The compounds **1**, **3**, and **5** showed significant inhibitory activity against the enzyme thrombin.

2. Results and Discussion. – The EtOH extract of the whole plant of *Duranta repens* was partitioned between H_2O and $CHCl_3$. Fractionation of the $CHCl_3$ fraction by MPLC, column chromatography, and TLC afforded compounds **1**–**5** as described in the *Exper. Part*.

Compound **1** was assigned the molecular formula $C_{22}H_{24}O_8$ by HR-EI-MS (M^+ at m/z 416.1477). It gave a red colour in the *Shinoda* test [7], typical for a flavonoid. Negative results in the *Quastel* test [8] indicated the absence of an *ortho*-dihydroxy moiety. The UV spectrum, with λ_{max} at 273 and 343 cm^{-1}, suggests **1** is a flavonoid [9]. The IR spectrum revealed the presence of an α,β-unsaturated C=O (1655 and 1595 cm^{-1}), a MeO (2900 and 1193 cm^{-1}), and OH groups (3385 cm^{-1}). The ^{1}H-NMR of **1** showed a striking resemblance to that of aliarin [7], indicating the same



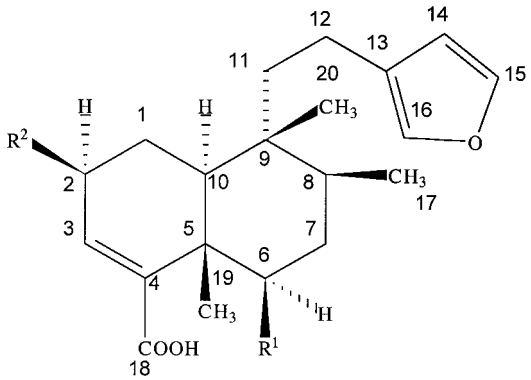
1 $R^1 = R^4 = R^5 = H$, $R^2 = R^3 = Me$, $R^6 = HOCH_2CH(Me)CH_2CH_2(1'')$

1a $R^1 = R^4 = R^5 = Ac$, $R^2 = R^3 = Me$, $R^6 = AcOCH_2CH(Me)CH_2CH_2(1'')$

2 $R^1 = R^4 = H$, $R^2 = R^3 = R^5 = Me$, $R^6 = HOCH_2CH(Me)CH_2CH_2(1'')$

2a $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = Me$, $R^6 = AcOCH_2CH(Me)CH_2CH_2(1'')$

5 $R^1 = R^2 = R^5 = R^6 = H$, $R^3 = R^4 = Me$



3, $R^1 = OH$, $R^2 = H$

4, $R^1 = H$, $R^2 = OH$

substitution pattern in rings A/B and the side chain (side chain: δ 3.45, 2.65, 1.66, 1.40, and 0.99; ring B: *ABX* at δ 7.84, 7.78, 6.85; 2 MeO groups at δ 3.87, 3.74). The ^{13}C-NMR spectrum (BB and DEPT) of **1** corroborated the presence of 3 Me, 3 CH_2, 5 CH, and 11 quaternary C-atoms, with a resonance for C(8) (δ 95.1) typical of 5,6,7-oxygenated flavonols [10]. Acetylation of **1** gave the tetraacetate **1a**, whose spectra confirmed the structure assignment of **1** as 3,7,4′-trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5,6-dimethoxyflavone.

The mass spectrum of **1** showed peaks at *m/z* 416 (M^+),343 ([$M - C_4H_9O$]$^+$), 342 ([$M - C_4H_8O$]$^+$), 329 ([$M - C_4H_8O - CH_3$]$^+$), 207 ([$M - C_{10}H_9O_5$]$^+$), and 152 ([$M - C_{14}H_{16}O_5$]$^+$). These fragments confirmed the presence of two MeO and 1 OH group in ring A of **1**, 1 OH and the side chain in ring B, and the remaining OH at C(3) [11]. Occurrence of a benzyl cleavage explained the loss of 73 a.m.u., while the loss of 72 a.m.u. was due to the β-cleavage of the side chain with H-transfer to the aromatic nucleus *via* a 1,6 rearrangement. Since this required at least one free *ortho* position [12], the side chain had to be located at either C(3′) or C(4′). The position of the side chain at C(3′) was confirmed by an HMBC experiment in which the CH_2(1″) protons of the side chain at δ 2.65 showed ^2J correlation to C(3′) (δ 131.46) and ^3J interaction with C(2′) (δ 130.8) and C(4′) (δ 158.6). The 5,6,7-oxygenation pattern was also confirmed by ^2J correlations of H–C(8) (δ 6.48) to C(7) (δ 158.5) and C(9) (δ 153.78) and ^3J interaction of H–C(8) to C(6) (δ 132.06) and C(10) (δ 106.4).

Compound **2** was assigned the molecular formula $C_{23}H_{26}O_8$ by HR-EI-MS (M^+ at *m/z* 430.1629). It gave a positive *Shinoda* and a negative *Quastel* test for flavonoids [7][8]. The IR and ^{1}H- and ^{13}C-NMR data of **2** resembled closely those of **1**. The detailed ^{1}H-NMR study revealed the same substitution pattern in rings A, B, and C, except for the presence of one additional MeO group. Acetylation provided a triacetate **2a** confirming the presence of three OH groups. Compound **2** was, therefore, assigned the structure 3,7-dihydroxy-3′-(4-hydroxy-3-methylbutyl)-5,6,4′-trimethoxyflavone.

The ^{13}C-NMR spectrum (BB and DEPT) of **2** revealed the presence of 4 Me, 3 CH_2, 5 CH, and 11 quaternary C-atoms, as well as the same substitution pattern in rings A/B and the side chain as found for **1**. The MS of **2** exhibited peaks at *m/z* 430 (M^+), 357 ([$M - C_4H_9O$]$^+$), 356 ([$M - C_4H_8O$]$^+$), 343 ([$M - C_4H_8O - CH_3$]$^+$), 221 ([$M - C_{10}H_9O_5$]$^+$), and 164 ([$M - C_{14}H_{18}O_5$]$^+$). The fragmentation pattern established that 2 MeO and 1 OH group were at ring A, 1 MeO group and the side chain at ring B, and the remaining OH was at C(3). The position of the side chain at C(3′) was determined by an HMBC experiment in which the CH_2(1″) protons of the side chain at δ 2.67 showed ^2J correlation to C(3′) (δ 130.8) and ^3J interaction with C(2′) (δ 129.5) and C(4′) (δ 156.6). Compound **2** differs from **1** in having a MeO group at ring B instead of an OH group. Its position at C(4′) was confirmed by HMBC exhibiting a ^3J correlation of the MeO at C(4′) (δ 3.81) to C(4′) (δ 156.6).

The HR-EI-MS of **3** provided the M^+ peak at *m/z* 332.1990, indicating the molecular formula $C_{20}H_{28}O_4$. The peaks at *m/z* 95, 94, and 81 suggested the presence of furan ring with an alkyl chain in **3** [13]. The UV spectrum showed an absorption at λ_{max} 212 nm, and the IR spectrum revealed the presence of an OH group (3336 cm^{-1}), unsaturation (1680 cm^{-1}), and a furan ring (1505 and 876 cm^{-1}). The ^{1}H-NMR spectrum of **3** closely resembled those of *trans*-clerodanes, revealing the same substitution pattern in rings A and B [14]. The ^{1}H- and ^{13}C-NMR, HMBC and NOE data of **3** allowed the assignment of the structure of 6β-hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic acid (relative configuration).

The ^{1}H-NMR spectrum of **3** exhibited signals for 2 tertiary Me (δ 0.70, 1.17) and 1 secondary Me group (δ 0.82, $J = 6.6$ Hz) and for an olefinic H–C(3) (*dd* at δ 7.01, $J = 3$, 4.7 Hz). A *dd* at δ 3.63 ($J = 4.8$, 11.0 Hz, 1 H), was assigned to the geminal proton of a secondary OH group. Typical low-field signals in the ^{1}H-NMR spectrum of **3** at δ 6.20, 7.15, and 7.30 were attributed to H–C(14), H–C(16), and H–C(15), respectively, suggesting the presence of a 3-substituted furan ring. The ^{13}C-NMR spectrum (BB and DEPT) corroborated the presence of 3 Me, 5 CH_2, 7 CH, and 5 quaternary C-atoms. The ^{13}C-NMR chemical shift of Me(19) was observed at δ 16.5 and the β-positioned axial Me(20) appeared at δ 17.2, while the β-positioned equatorial Me(17) resonated at δ 15.2. These values revealed the *trans* configuration at the A/B ring junction of **3** [15]. The position of the OH group at C(6) was confirmed by HMBC experiments, which showed ^2J correlation of Me(19) (δ 1.17) to C(5) (δ 45.4) and ^3J correlation to C(6) (δ 74.19), C(4) (δ 140.7), and C(10) (δ 45.4). The position of a COOH group at C(4) was also confirmed by HMBC experiments that showed ^2J correlation of H–C(3) (δ 7.0) to C(4) (δ 140.7) and C(2) (δ 27.8) and ^3J correlation to C(5) (δ 45.4) and C(18) (δ 172.2). The *AB* pattern centered at δ 1.56 and 1.64 could be assigned to CH_2(11); the HMBC showed ^2J correlation to C(9) (δ 38.4) and C(12) (δ 17.2) and ^3J correlation

to C(8) (δ 33.6), C(10) (δ 45.4), and C(20) (δ 17.2), confirming the attachment of the alkyl chain at C(9). A series of nuclear *Overhauser* effect (NOE) experiments carried out on **3** established NOEs between Me(19) and Me(20) and between Me(17) and Me(20), consistent with a *cis* relationship between these Me groups. These results and the finding that irradiation of H–C(10) did not cause any increase in the intensities of either the Me(19) or Me(20) signals confirmed the *trans* configuration of the A and B rings of the decalin system of **3** [14]. No NOE was observed between H–C(6) and both Me(19) and Me(20), indicating their *trans* relationship and establishing the β-configuration and equatorial conformation of OH–C(6). This was also confirmed by the coupling constants of H–C(6) and the inspection of the *Dreiding* model.

The HR-EI-MS of compound **4** provided the M^+ at m/z 332.1983 indicating the molecular formula $C_{20}H_{28}O_4$. As for **3**, the peaks at m/z 95, 94, and 81 were typical of an alkyl-substituted furan moiety [13]. The UV spectrum showed an absorption at λ_{max} 204 nm, and the IR spectrum revealed the presence of an OH group (3336 cm^{-1}), unsaturation (1680 cm^{-1}), and a furan ring (1505, 874 cm^{-1}). A comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** with those of **3** revealed a close similarity between the two compounds. The ^{1}H- and ^{13}C-NMR, HMBC, and NOE data were consistent with the structure of 2β-hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic acid for **4** (relative configuration).

The ^{1}H-NMR spectrum of **4** showed a signal at δ 4.30 (*m*, 1 H) indicating that one of the two protons at C(2) of **4** had been substituted by an OH group [16]. The ^{13}C-NMR spectrum (BB and DEPT) corroborated the presence of 3 Me, 5 CH_2, 7 CH, and 5 quaternary C-atoms. The ^{13}C-NMR showed the downfield shift of the C(2) signal at (δ 69.91) due to the OH group. The position of the OH group at C(2) was further confirmed by HMBC experiments, which showed ^2J correlation of H–C(3) (δ 6.11) to C(4) (δ 140.2) and C(2) (δ 69.91) and ^3J correlation of H–C(3) to C(18) (δ 170.0) and C(5) (δ 39.1). The coupling constant of H–C(2) was consistent with the β and equatorial orientation of the OH group. Further confirmation of the structure of **4** was provided by NOEs establishing the spatial proximity of H–C(2) and H–C(10). Other features of the 1D and 2D NMR spectra were similar to those of **3**.

Thrombotic vascular disease is a major cause of morbidity and mortality in the industrial world. The problems associated with established clinical thrombin inhibitors are well-recognized [17]. Considerable efforts are being focused on the development of safe new classes of selective inhibitors derived from both natural [18] and synthetic sources [19][20]. In the course of this work, we evaluated compounds **1**–**5** along with the reference compound leupeptin *in vitro* against bovine thrombin and trypsin. The compounds **2** and **4** were found to be inactive, while **3** and **5** showed a broad range of binding affinities or IC_{50} values. Compound **1** exhibited insignificant activity against trypsin (see *Table*). Compound **3** represents the lead prototype in this series. *Fig. 1* and *2* show the selectivity profiles of the inhibitors **3**, **1**, and **5**. Compound **3** was, therefore, identified as an active-site inhibitor of thrombin. Although **2** and **4** are similar in structure to **3**, they do not display significant *in vitro* activity, revealing the significance of functional groups around phenyl moieties for potency.

Table 1. *Enzyme Selectivity* (IC_{50} values [mM]) *of Natural Compounds from* Duranta repens *for Thrombin and Trypsin*

		1	**3**	**5**	Leupeptin
IC_{50} [mM]:	Thrombin	0.1 ± 0.021	0.084 ± 0.04	0.39 ± 0.01	0.0454 ± 0.03
	Trypsin	–	0.92 ± 0.061	0.80 ± 0.21	0.0258 ± 0.001

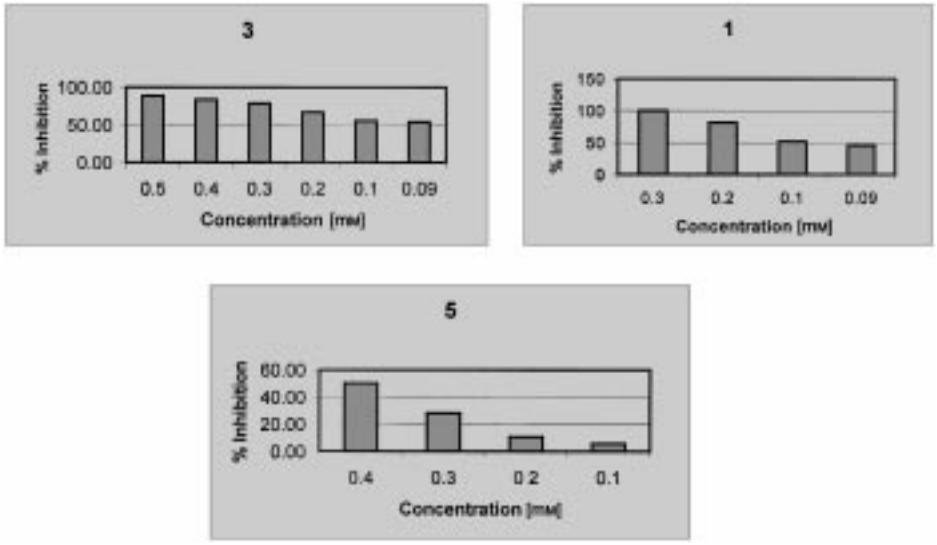


Fig. 1. *Inhibition* [%] *of thrombin by* **3**, **1**, *and* **5** *at various concentrations*

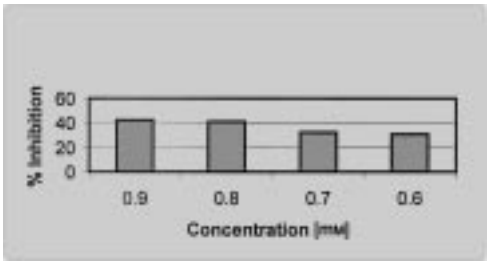


Fig. 2. *Inhibition of trypsin by* **3** *at various concentrations*

Experimental Part

General. Column chromatography (CC): silica gel, 70–230 mesh. Flash chromatography (FC): silica gel, 220–440 mesh. TLC: precoated silica gel *G-25-UV*$_{254}$ plates; detection at 254 and 366 nm and by *Dragendorff*'s reagent. Optical rotations: *Jasco DIP-360* polarimeter. UV Spectra: *Hitachi UV-3200* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco 302-A* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR, COSY, HMQC, and HMBC Spectra: *Bruker* spectrophotometers, at 500 and 400 MHz; chemical shifts δ in ppm rel. to $SiMe_4$ as internal standard and coupling constants *J* in Hz. EI-, FAB-, and HR-EI-MS: *JMS-HX-110* with a data system and *JMS-DA-500* mass spectrometers; *m*/*z* (rel. int).

Plant Material. The whole plant of *Duranta repens* (Verbenaceae), collected from District Chitral, N.W.F.P (Pakistan), in April, 1997, was identified by Prof. *M. Qaiser*, Department of Botany, University of Karachi. A voucher specimen was deposited in the herbarium of the University of Karachi.

Extraction and Isolation. The shade-dried ground plant (20 kg) was exhaustively extracted with EtOH at r.t. The combined EtOH extract was evaporated to a dark greenish mass which was partitioned initially between hexane/H_2O and then $CHCl_3$/H_2O. The $CHCl_3$-soluble fraction was subjected to MPLC (hexane, hexane/$CHCl_3$, $CHCl_3$, and $CHCl_3$/MeOH, in increasing order of polarity). The fraction obtained from pure $CHCl_3$ was rechromatographed (silica gel; hexane/acetone in increasing order of polarity). The fractions obtained from hexane/acetone 90:10, 85:15, and 80:20 showing similar TLCs were combined and submitted to CC (hexane/

AcOEt 1:1 and 4:6): five major compounds. Compounds **3** and **5** were purified by rechromatography (silica gel, hexane/AcOEt 1:1). Compounds **1**, **2**, and **4** were purified by prep. TLC (hexane/acetone 65:35, 70:30, and hexane/AcOEt/MeOH 60:39:1, resp.). R_f values for **1**–**5** (see below) were determined for the eluent hexane/AcOEt/MeOH 60:39:1.

Enzyme Assays. Enzymatic activity of bovine thrombin (*Sigma*) was measured in a buffer containing 0.145M NaCl, 0.005M KCl, 1 mg/ml polyethyleneglycol (PEG-8000), 0.030M HEPES (pH 7.4), and 0.096 U/well final concentration in the microtiter-plate-based assay. The enzyme was incubated at 37° with the inhibitor for 15 min before starting the reaction with 0.5 mM *N*-benzoyl-Phe-Val-Arg *p*-nitroanilide. Time-dependent optical-density change was followed at 405 nm with a microplate reader (*Molecular Devices SPECTRA* max *340*) at 37°. The IC_{50} values were the average of at least three determinations performed in triplicate. A stock soln. of the inhibitor in DMSO was diluted to the desired range of concentrations.

Bovine pancreatic trypsin (*Sigma*) was assayed as for thrombin, except that the buffer was 50 mM *Tris* · HCl (pH 7.5), and the reaction was started with 1 mM *N*α-benzoyl-DL-Arg *p*-nitroanilide.

3,7,4′-Trihydroxy-3′-(4-hydroxy-3-methylbutyl)-5,6-dimethoxyflavone (= *3,7-Dihydroxy-2-[4-hydroxy-3-(4-hydroxy-3-methylbutyl)phenyl]-5,6-dimethoxy-4*H-*1-benzopyran-4-one*; **1**). Gummy solid (40 mg). R_f 0.40. $[\alpha]_D^{25} = +110$ ($c = 0.01$, MeOH). UV (MeOH): 273 (8.06), 343 (8.02). IR (KBr): 3385, 2900, 1655, 1595, 1193. ^{1}H-NMR ($CDCl_3$/CD_3OD, 400 MHz): 6.48 (*s*, H−C(8)); 7.84 (*d*, *J* = 2.3, H−C(2′)); 6.85 (*d*, *J* = 8.5, H−C(5′)); 7.78 (*dd*, *J* = 8.5, 2.3, H−C(6′)); 2.65 (*t*, $CH_2$1″)); 1.40 (*m*, CH_2(2″)); 1.66 (*m*, H−C(3″)); 3.45 (*d*, *J* = 6.4, HOCH_2(4″)); 0.99 (*d*, *J* = 6.65, Me−C(3″)); 3.87 (*s*, MeO−C(5)); 3.74 (*s*, MeO−C(6)). ^{13}C-NMR ($CDCl_3$/CD_3OD, 125 MHz): 153.65 (C(2)); 139.09 (C(3)); 180.2 (C(4)); 159.6 (C(5)); 132.06 (C(6)); 158.5 (C(7)); 95.15 (C(8)); 153.78 (C(9)); 106.4 (C(10)); 122.4 (C(1′)); 130.8 (C(2′)); 131.46 (C(3′)); 158.6 (C(4′)); 116.4 (C(5′)); 128.9 (C(6′)); 28.7 (C(1″)); 34.5 (C(2″)); 36.7 (C(3″)); 68.4 (C(4″)); 17.27 (*Me*−C(3″)); 61.15 (*Me*O−C(5)); 60.7 (*Me*O−C(6)). HR-FAB-MS (pos.): 417.1476. HR-EI-MS: 416.1477 ($C_{22}H_{24}O_8^+$, M^+; calc. 416.1471). EI-MS: 416 (100), 401 (32.38), 398 (8.82), 373 (4), 343 (6), 342 (3.2), 329 (6.2), 207 (1), 152 (2.6).

Tetraacetate **1a**. Compound **1** (20 mg) was acetylated with Ac_2O (3 ml) in pyridine (1 ml) at r.t. for 24 h. Usual workup and prep. TLC afforded **1a** (14 mg). Gummy solid. ^{1}H-NMR ($CDCl_3$/CD_3OD, 400 MHz): 0.95 (*d*, *J* = 6.8, Me−C(3″)); 1.92 (*s*, AcO); 2.36 (*s*, AcO); 2.20 (*s*, AcO); 2.22 (*s*, AcO).

3,7-Dihydroxy-3′-(4-hydroxy-3-methylbutyl)-5,6,4′-trimethoxyflavone (= *3,7-Dihydroxy-2-[3-(4-hydroxy-3-methylbutyl)-4-methoxyphenyl]-5,6-dimethoxy-4*H-*1-benzopyran-4-one*; **2**). Gummy solid (35 mg). R_f 0.56. $[\alpha]_D^{25} = +73.6$ ($c = 0.02$, MeOH). UV (MeOH): 271 (8.25), 340 (8.33). IR (KBr). 3380, 2905, 1650, 1590, 1190. ^{1}H-NMR ($CDCl_3$/CD_3OD, 400 MHz); 6.56 (*s*, H−C(8)); 7.98 (*d*, *J* = 2.3, H−C(2′)); 7.12 (*d*, *J* = 8.5, H−C(5′)); 7.96 (*dd*, *J* = 8.5, 2.3, H−C(6′)); 2.67 (*t*, H−C(1″)); 1.40 (*m*, H−C(2″)); 1.65 (*m*, H−C(3″)); 3.46 (*d*, *J* = 6.4, HOCH_2(4″)); 0.98 (*d*, *J* = 6.67, Me−C(3″)); 3.84 (*s*, MeO−C(5)); 3.69 (*s*, MeO−C(6)); 3.81 (*s*, MeO−C(4′)). ^{13}C-NMR ($CDCl_3$/CD_3OD, 125 MHz): 152.06 (C(2)); 138.19 (C(3)); 178.89 (C(4)); 159.6 (C(5)); 131.3 (C(6)); 156.4 (C(7)); 93.9 (C(8)); 152.38 (C(9)); 105.5 (C(10)); 122.23 (C(1′)); 129.510 (C(2′)); 130.87 (C(3′)); 156.6 (C(4′)); 110.04 (C(5′)); 127.9 (C(6′)); 27.47 (C(1″)); 33.01 (C(2″)); 35.4 (C(3″)); 67.7 (C(4″)); 16.49 (*Me*−C(3″)); 60.68 (*Me*O−C(5)); 60.07 (*Me*O−C(6)); 55.4 (*Me*O−C(4′)). HR-FAB-MS (pos.): 431.1630 ([*M* + H]$^+$). HR-EI-MS: 430.1629 ($C_{23}H_{26}O_8^+$, M^+; calc. 430.1627). EI-MS: 430 (100), 415 (33.5), 412 (12), 387 (11.16), 357 (7), 356 (5), 343 (12), 221 (3.2), 171 (15), 164 (1.5), 69 (21).

Triacetate **2a**. Compound **2** (18 mg) was acetylated with Ac_2O (3 ml) in pyridine (1 ml) at r.t. for 24 h. Usual workup and prep. TLC afforded **2a** (13 mg). Gummy solid. ^{1}H-NMR ($CDCl_3$/CD_3OD, 400 MHz): 0.96 (*d*, *J* = 6.75, Me−C(3″)); 1.93 (*s*, AcO); 2.35 (*s*, AcO); 2.19 (*s*, AcO); 2.23 (*s*, AcO).

6β-Hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic Acid (= rel-*(4a*R,5S,7R,8S,8*a*R)-*8-[2-(Furan-3-yl)ethyl]-1,2,4a,5,6,7,8,8a-octahydro-5-hydroxy-4a,7,8-trimethylnaphthalene-4-carboxylic Acid*; **3**). Gummy solid (40 mg). R_f 0.16 $[\alpha]_D^{25} = -18.2$ ($c = 0.05$, MeOH). UV ($CHCl_3$): 212 (8.26). IR ($CHCl_3$): 3336, 3160, 1680, 1603, 1505, 876. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.51 (*m*, CH_2(1)); 1.68 (*m*, CH_2(1′)); 2.19 (*m*, CH_2(2)); 2.30 (*m*, CH_2(2′)); 7.01 (*dd*, *J* = 3, 4.7, H−C(3)); 3.63 (*dd*, *J* = 4.8, 11.0, H−C(6)); 1.62 (*m*, CH_2(7)); 1.32 (br. *d*, *J* = 12, H−C(10)); 1.56 (*ddd*, *J* = 14.2, 12.1, 5.0, H−C(11)); 1.64 (*ddd*, *J* = 14.2, 12.1, 5.2, H−C(11′)); 2.15 (*ddd*, *J* = 14.2, 12.1, 5.2, H−C(12)); 2.25 (*ddd*, *J* = 14.2, 12.1, 5.0, H−C(12′)); 6.20 (*dd*, *J* = 0.8, 1.7, H−C(14)); 7.30 (*t*, *J* = 1.67, H−C(15)); 7.15 (*dd*, *J* = 0.9, 1.4, H−C(16)); 0.82 (*d*, *J* = 6.6, Me(17)); 1.17 (*s*, Me(19)); 0.70 (*s*, Me(20)). ^{13}C-NMR ($CDCl_3$, 125 MHz): 17.0 (C(1)); 27.8 (C(2)); 142.04 (C(3)); 140.79 (C(4)); 45.4 (C(5)); 74.19 (C(6)); 35.5 (C(7)); 33.6 (C(8)); 38.4 (C(9)); 45.4 (C(10)); 38.4 (C(11)); 17.2 (C(12)); 125.07 (C(13)); 110.64 (C(14)); 142.4 (C(15)); 138.0 (C(16)); 15.2 (C(17)); 172.2 (C(18)); 16.5 (C(19)); 17.2 (C(20)). HR-FAB-MS (pos.): 333.1991. HR-EI-MS: 332.1990 ($C_{20}H_{28}O_4^+$, M^+; calc. 332.1987). EI-MS: 332 (15), 314 (18), 219 (60), 201 (18), 173 (12), 125 (18), 95 (70), 94 (13), 81 (100).

2β-Hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic Acid (= rel-*(2*R,*4a*R,*7*R,*8*S,*8a*R*)-8-[2-(Furan-3-yl)ethyl]-1,2,4a,5,6,7,8,8a-octahydro-2-hydroxy-4a,7,8-trimethylnaphthalene-4-carboxylic Acid*; **4**). Gummy solid (25 mg). R_f 0.24. $[\alpha]_D^{25} = +100$ ($c = 0.02$, MeOH). UV (MeOH): 204 (8.6). IR (KBr): 3336, 3160, 1680, 1602, 1560, 1505, 874. ^{1}H-NMR ($CDCl_3/CD_3OD$, 400 MHz); 2.1 (*m*, H−C(1)); 1.43 (*m*, H−C(1′)); 4.30 (*m*, H−C(2)); 6.11 (br. *s*, H−C(3)); 1.17 (*m*, H−C(6)); 2.32 (*m*, H−C(6′)); 1.43 (*m*, H−C(7)); 1.5 (*m*, H−C(7′)); 1.59 (*m*, H−C(8)); 1.38 (br. *d*, $J = 12$, H−C(10)); 1.57 (*ddd*, $J = 14.4$, 12.4, 5.0, H−C(11)); 1.66 (*ddd*, $J = 14.4$, 12.4, 5.2, H−C(11′)); 2.19 (*ddd*, $J = 14.4$, 12.4, 5.2, H−C(12)); 2.32 (*ddd*, $J = 14.4$, 12.4, 5.0, H−C(12′)); 6.26 (*dd*, $J = 0.78$, 1.7, H−C(14)); 7.32 (*t*, $J = 1.68$, H−C(15)); 7.2 (br. *s*, H−C(16)); 0.82 (*d*, $J = 6.5$, H−C(17)); 1.22 (*s*, H−C(19)); 0.77 (*s*, H−C(20)). ^{13}C-NMR ($CDCl_3/CD_3OD$, 400 MHz): 29.0 (C(1)); 69.9 (C(2)); 133.5 (C(3)); 140.2 (C(4)); 39.1 (C(5)); 37.2 (C(6)); 28.3 (C(7)); 37.4 (C(8)); 39.6 (C(9)); 46.5 (C(10)); 36.8 (C(11)); 19.2 (C(12)); 126.8 (C(13)); 111.9 (C(14)); 143.9 (C(15)); 139.6 (C(16)); 16.6 (C(17)); 170.0 (C(18)); 21.5 (C(19)); 19.1 (C(20)). HR-FAB-MS (pos.): 333.1987. HR-EI-MS: 332.1983 ($C_{20}H_{28}O_4^+$, M^+; calc. 332.1987). EI-MS: 332 (12), 314 (15), 219 (56), 201 (12), 173 (12), 125 (17), 95 (67), 94 (15), 81 (100).

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