α-Glucosidase Inhibitory Constituents from *Duranta repens*

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Three *C*-alkylated flavonoids 7-*O*-α-D-glucopyranosyl-3,5-dihydroxy-3′-(4″-acetoxyl-3″-methylbutyl)-6,4′-dimethoxyflavone (1), 7-*O*-α-D-glucopyranosyl-3,4′-dihydroxy-3′-(4″-acetoxyl-3″-methylbutyl)-5,6-dimethoxyflavone (2), 3,7,4′-trihydroxy-3′-(8″-acetoxy-7″-methyloctyl)-5,6-dimethoxyflavone (3) and a *trans*-clerodane type diterpenoid (−)-6β-hydroxy-5β,8β,9β,10α-cleroda-3,13-dien-16,15-olid-18-oic acid (4) are reported from *Duranta repens* along with (+)-hardwickiic acid (5) and (+)-3,13-clerodadien-16,15-olid-18-oic acid (6), isolated for the first time from this species. Their structures were established on the basis of the spectral methods, especially two dimensional (2D) NMR spectroscopy.

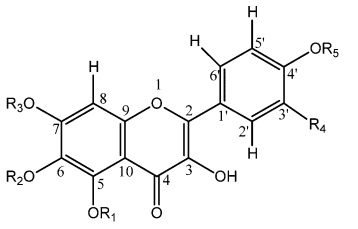
Key words Verbenaceae; *C*-alkylated flavonoid; *trans*-clerodane; α-glucosidase inhibition

Enzyme inhibitors play a significant role in preservation of human health. In our search for plant based enzyme inhibitory constituents we have previously reported a number of naturally occurring compounds which may be used in the treatment of a variety of ailments.[1—3] In continuation of these studies, we report the isolation and structural elucidation of α-glucosidase inhibitory *C*-alkylated flavonoids (**1**—**3**) and a *trans*-clerodane type diterpenoid (**4**) from *Duranta repens* (Verbenaceae). (+)-Hardwickiic acid (**5**) and (+)-3,13-clerodadien-16,15-olid-18-oic acid (**6**) are also reported for the first time from this species. This plant grows in northern parts of Pakistan and various medicinal properties are attributed to it.[4,5] Previous studies on this species have resulted in the isolation of diterpenoids, flavonoids, steroid and coumarinolignoids.[6,7]

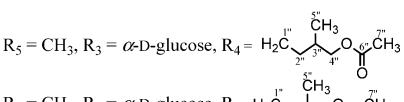
Results and Discussion

The methanolic extract of *Duranta repens* showed strong cytotoxicity in brine shrimp lethality test.[8] The fractionation and subsequent screening revealed strong α-glucosidase inhibitory activity in the chloroform fraction. Bioassay directed isolation studies on this fraction have resulted in the isolation of compounds (**1**—**6**) as described in the experimental.

Compound **1** was isolated as a yellowish gummy solid and its molecular formula was established as $C_{30}H_{36}O_{14}$ by a $[M+H]^+$ peak at *m/z* 621.2174 in high resolution fast atom bombardment mass spectrometry (HR-FAB-MS). Positive Shinoda test,[9] negative Quastel test indicated the absence of an *ortho* dihydroxyl moiety[10] and the UV absorption maxima in MeOH at 273 and 343 nm suggested that compound **1** was a flavonoid.[11] The IR spectrum showed the aromatic ring at 1597 cm^{-1}, α,β-unsaturated carbonyl group at 1656 cm^{-1}, methoxyl group at 2925 and 1190 cm^{-1} and hydroxyl group at 3396 cm^{-1}. The ^{1}H-NMR spectrum of **1** exhibited an ABX system in ring B of flavonoid resulting in signals at δ 6.87 (1H, d, *J*=8.5 Hz, H-5′), 7.78 (1H, dd, *J*=8.5, 1.9 Hz, H-6′) and 7.86 (1H, d, *J*=1.9 Hz, H-2′). A singlet at δ 6.44 could be assigned to the aromatic proton of ring A.[9] The signals of two methoxyl groups appeared at δ 3.84 (3H, s, MeO-4′) and 3.77 (3H, s, MeO-6) while the benzylic methylene protons of ring B resonated at δ 2.65 (2H, t, *J*=7.2 Hz, H-1″). The signals of the side chain were observed at δ 0.99 (3H, d, *J*=6.7 Hz, H-5″), 1.42 (2H, m, H-2″), 1.66 (1H, m, H-3″), 1.89 (3H, s, H-7″) and 3.48 (2H, d, *J*=6.5 Hz, H-4″) which identified the side chain as 4″-acetoxy-3″-



1 R_1 = H, R_2 = R_5 = CH_3, R_3 = α-D-glucose, R_4 =



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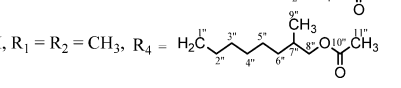

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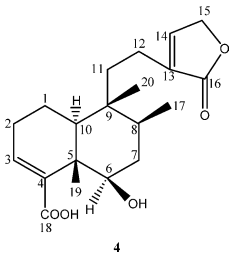

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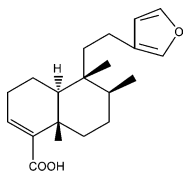

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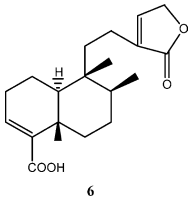

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methylbutyl. The presence of sugar was revealed by a doublet of anomeric proton at δ 5.30 (1H, d, J=3.7 Hz, H-1‴). The signals of four protons geminal to hydroxyl groups appeared at [δ 3.55 (1H, t, J=9.5 Hz, H-4‴), 3.68 (1H, m, H-5‴), 3.85 (1H, t, J=9.5 Hz, H-3‴), 3.98 (1H, dd, J=9.5, 3.7 Hz, H-2‴)] and methylene protons at δ 3.66 (1H, dd, J=11.2, 4.5 Hz, H-6‴a) and 3.76 (1H, dd, J=11.2, 5.1 Hz, H-6‴b). The small coupling constant at H-1‴ allowed us to assign α-configuration to the glucose moiety. The glucose moiety could be identified through acid hydrolysis, provided the glycone which could be identified as D-glucose through sign of its optical rotation and comparison of retention time of its TMS ether with that of standard in GLC. The electron impact mass spectrometry (EI-MS) spectrum showed a strong fragment peak at m/z 416 due to the loss of sugar and acetyl moieties from the molecule. The other characteristic fragments originated from this peak which were evident by link scan measurement at m/z 344 ([416−C_4H_8O]$^+$), 343 ([416−C_4H_9O]$^+$), 221 ([416−$C_9H_7O_5$]$^+$), 182 ([416−$C_{14}H_{18}O_3$]$^+$) and 138 ([416−$C_{15}H_{18}O_5$]$^+$). The fragment at m/z 138 ([416−$C_{15}H_{18}O_5$]$^+$) supported the presence of one methoxyl and two hydroxyl groups on ring A[9] and the fragment at m/z 221 ([416−$C_9H_7O_5$]$^+$) confirmed the attachment of methoxyl and side chain on ring B and hydroxyl at C-3 position.[6] The broad band (BB) and distortionless enhancement by polarization transfer (DEPT) ^{13}C-NMR spectra of **1** showed a total of 30 carbons with the signal of anomeric carbon at δ 99.9 and acetyl carbons at δ 23.2 and 175.2. The assignments of all carbons were achieved by heteroatom multiple quantum coherence (HMQC). In heteronuclear multiple bond connectivity (HMBC) experiment, the anomeric proton at δ 5.30 showed a ^3J correlations to C-7 (δ 156.1) and C-5‴ (δ 74.1). This confirmed the attachment of sugar moiety at C-7 position. The ^2J and ^3J connectivities of H-1″ at δ 2.65 with C-3′ (δ 131.1), C-2″ (δ 34.2), C-2′ (δ 130.2) and C-4′ (δ 159.2) confirmed the position of side chain at C-3′. The carbons at position C-5, C-7 and C-9 were assigned through HMQC and HMBC experiments. The H-8 proton at δ 6.44 showed ^2J correlations with C-7 (δ 156.1) and C-9 (δ 153.6) and ^3J correlations with C-6 (δ 130.6) and C-10 (δ 106.5). It didnot show correlation with C-5. The methoxyl group at δ 3.77 exhibited ^3J correlation with C-6 (δ 130.6), thereby confirming its attachment to C-6. The position of other methoxyl group at C-4′ and attachment of acetyl moiety at C-4″ could also be inferred through HMBC correlations (Fig. 1). Thus compound **1** was assigned the structure 7-*O*-α-D-glucopyranosyl-3,5-dihydroxy-3′-(4″-acetoxy-3″-methylbutyl)-6,4′-dimethoxyflavone.

Compound **2** was obtained as a yellowish gummy solid and showed [M+H]$^+$ peak in positive mode HR-FAB-MS at m/z 621.2175 corresponding to molecular formula $C_{30}H_{36}O_{14}$. The UV, IR, NMR and MS spectra closely resembled to those of **1**. The difference between these two compounds were the interchanged position of methoxyl and hydroxyl functionalities at C-5 and C-4′ position. Acid hydrolysis of **2** provided D-glucose. The mass fragmentation pattern differed from **1** in having fragments at m/z 152 ([416−$C_{14}H_{16}O_5$]$^+$) and 207 ([416−$C_{10}H_9O_5$]$^+$) indicating the presence of two methoxyl groups in ring A and one hydroxyl and the side chain in ring B.[6] The structure was further confirmed by HMBC correlations as shown in Fig. 2. The proton

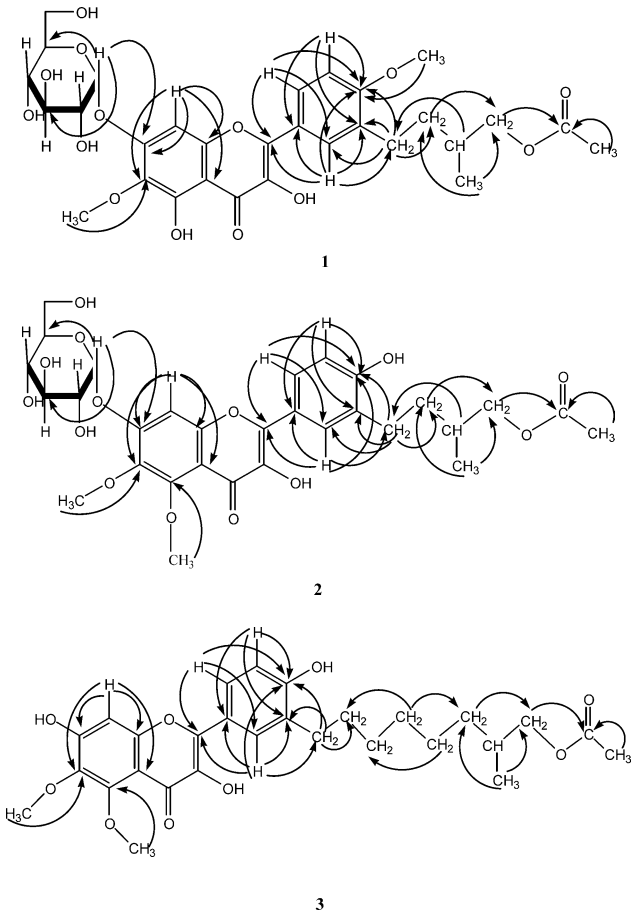

Fig. 1. Important HMBC Interactions of Compounds **1**, **2** and **3**

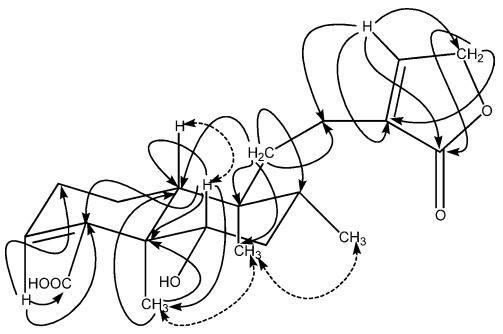

Fig. 2. Stereostructure of **4** with NOE and HMBC Correlations
HMBC interactions, NOE interactions.

at δ 3.81 showed ^3J correlation with C-5 (δ 158.5) and the proton at δ 3.76 showed ^3J correlation with C-6 (δ 132.1), thereby confirming the positions of the two methoxyl groups at C-5 and C-6, respectively. The oxygenated quaternary carbon at δ 158.9 in ring B should, therefore, be assigned to C-4′, since it showed interactions with H-2′, H-5′ and H-6′, respectively. This allowed us to assign hydroxyl group to position C-4′. The 5,6,7-oxygenation pattern was also confirmed by ^2J correlations of H-8 at δ 6.45 with C-7 (δ 157.4) and C-9 (δ 153.6) and ^3J interactions with C-6 (δ 132.1) and C-10 (δ 106.6). Further HMBC interactions were in accordance to the assigned structure of **2** as 7-*O*-α-D-glucopyranosyl-3,4′-

dihydroxy-3′-(4″-acetoxy-3″-methylbutyl)-5,6-dimethoxyflavone.

Compound **3** was isolated as yellowish gummy solid. The molecular formula $C_{28}H_{34}O_9$ was determined by $[M+H]^+$ ion at *m/z* 515.2270 in HR-FAB-MS. The IR, UV and NMR spectra of compound **3** were very similar to compound **2** except the presence of hydroxyl group instead of sugar moiety at C-7 position and the additional signals for C-11 side chain. The detailed study of ^{1}H-NMR revealed the same substitution pattern in rings A, B and C but the signals of side chain differed from **2**. Four protons multiplet at δ 1.48 was assigned to H-2″ and H-3″ and a further four protons multiplet at δ 1.54 to H-4″ and H-5″. Two protons multiplet at δ 1.60 was due to H-6″. The H-7″ appeared as a multiplet at δ 1.70. The H-8″ methylene protons appeared as doublet at δ 3.48 (J=6.6 Hz). Two methyl signals appeared at δ 1.01 and 1.92. The BB and DEPT ^{13}C-NMR spectrum of **3** corroborated the presence of twenty eight carbons: 4 methyl, 7 methylene, 5 methine and 12 quaternary carbons. The C-2″ methylene signal appeared at δ 30.1. The C-3″, C-4″ and C-5″ methylene showed signals at δ 32.2, 32.8 and 33.4, respectively. The carbonyl carbon of acetyl group appeared at δ 174.9. The chemical shifts at δ 158.8, 157.9 and 153.9 could be assigned to C-5, C-7 and C-9 respectively in analogy to compound **2**. The substitution pattern in rings A, B and C were similar to *C*-alkylated flavonoids.[6] The EI-MS spectrum of **3** exhibited an ion at *m/z* 472 ($[M-C_2H_2O]^+$). This ion disintegrated further to the following characteristic fragments at *m/z* 344 ($[C_{18}H_{16}O_3]^+$), 263 ($[C_{16}H_{23}O_3]^+$), 152 ($[C_8H_8O_3]^+$). The fragments ion at *m/z* 152 and 263 indicated the presence of two methoxyl and a hydroxyl groups in ring A, one hydroxyl group and the side chain in ring B and hydroxyl group at C-3 position, respectively.[6] The structure of compound **3** was further confirmed by HMQC and HMBC correlations shown in Fig. 3. The H-8 at δ 6.50 showed ^2J interactions with C-7 (δ 157.9) and C-9 (δ 153.9) and ^3J correlations with C-6 (δ 132.2) and C-10 (δ 107.0). The proton at δ 3.88 showed ^3J interaction with C-5 (δ 158.8) and proton at δ 3.78 showed ^3J correlation with C-6 (δ 132.2), thereby confirming the position of two methoxyl groups at C-5 and C-6, respectively. The compound **3** was thus assigned the structure 3,7,4′-trihydroxy-3′-(8″-acetoxy-7″-methyloctyl)-5,6-dimethoxyflavone.

Compound **4** was assigned the molecular formula $C_{20}H_{28}O_5$ on the basis of HR-EI-MS. The UV showed an absorption with λ_{max} at 212 nm and its IR spectrum showed a characteristic absorption of an α,β-unsaturated γ-lactone and an α,β-unsaturated carbonyl group at 1750 and 1685 cm^{-1}, respectively. The absorptions at 3336 and 2955 cm^{-1} indicated the presence of a hydroxyl and carboxyl groups, respectively. The EI-MS spectrum gave a $[M-H_2O]^+$ peak at *m/z* 330 due to the loss of water molecule. The ions at *m/z* 173, 201 and 219 showed the presence of diterpenoid skeleton.[12] The ^{1}H-NMR spectrum exhibited typical signals for tricyclic clerodane carbon skeleton including a secondary methyl at δ 0.82 (d, J=6.6 Hz, CH_3-17) and two tertiary methyls at δ 0.76 (s, CH_3-20) and 1.21 (s, CH_3-19). The olefinic proton showed signal at δ 6.84 (dd, J=3.0, 4.5 Hz, H-3). The geminal proton of a secondary hydroxyl group showed signal at δ 3.63 (dd, J=4.8, 11.0 Hz, H-6). The low-field signal at δ 7.15 (1H, t, J=1.5 Hz, H-14) indicated the presence of an α-substituted γ-butenolide.[12] The BB and DEPT ^{13}C-NMR of **4** corroborated the presence of three methyl, six methylene, five methine and six quaternary carbons. The chemical shift of CH_3-19 was observed at δ 16.6. The β-oriented and axial CH_3-20 appeared at δ 17.4 while β and equatorial CH_3-17 resonated at δ 15.6. These values revealed the *trans* configuration at the A/B ring junction in **4**[13] which could further be proved by nuclear Overhauser effect (NOE) correlations shown in Fig. 4. The ^{1}H–^{1}H and ^{1}H–^{13}C connectivities were supported by the correlation spectroscopy (^{1}H–^{1}H-COSY) and HMQC spectra. The position of the hydroxyl group could be assigned to C-6 by HMBC since interactions of H-6 (δ 3.63) with C-4 (δ 140.8), C-5 (δ 44.7), C-10 (δ 45.6) and CH_3-19 (δ 16.6) were observed. The H-14 at δ 7.15 showed ^3J correlations with C-12 (δ 18.8) and C-16 (δ 174.3). The oxy methylene protons at δ 4.77 showed ^3J correlations with C-13 (δ 134.9) and C-16 (δ 174.3) besides ^{1}H–^{1}H COSY interaction with H-14 (δ 7.15), thereby confirming their presence at C-15 and subsequently the carbonyl group at C-16 in α-substituted γ-butenolide. Further HMBC interactions are illustrated in Fig. 4. The relative stereochemistry of **4** was determined on the basis of NOE experiments. The methyl group (δ 1.21, CH_3-19) attached to C-5 was revealed as being close in space to the methyl group (δ 0.76, CH_3-20). Thus, these were concluded to be on the same side in axial orientation. The methyl protons at δ 0.82 (CH_3-17) were close to the methyl protons at δ 0.76 (CH_3-20) which showed that the C-17 methyl group was equatorial and C-20 methyl group was axial. Thus the *cis* relationship of all the three methyl groups were confirmed. No NOE was observed between H-10 and the methyl groups, indicating a *trans* stereochemistry of the A and B rings of the decalin system of **4**.[12] However, it showed signal enhancement for H-6 suggesting the latter to be axial. Irradiation of H-6 did not cause any increase in the intensities of CH_3-19 and CH_3-20 indicating their *trans* relationship and establishing the α-configuration and equatorial conformation of OH. This was also confirmed from the coupling constant and inspection of Drieding model.[6] Thus, compound **4** was established as (−)-6β-hydroxy-5β,8β,9β,10α-cleroda-3,13-dien-16,15-olid-18-oic acid.

The structure of the known compounds **5** and **6** were established by comparison of their spectral data with literature values.[12]

Non insulin dependent diabetes mellitus (NIDDM) is caused by a secretory decrease in insulin from pancreatic Langerhans β cells or lowering of the insulin resistance. In addition, the long term manifestation of this disease can result in the development of retinopathy, neuropathy, cataracts and so on. Among the therapeutic drugs to prevent a high blood glucose level, the inhibitors of α-glucosidase (AGH, EC 3.2.1.20), which is a membrane-bound enzyme at the epithelium of the small intestine that catalyzes the cleavage of glucose from disaccharide, are effective for delaying glucose absorption.[14] The inhibitors of α-glucosidase could retard the use of dietary carbohydrates to suppress postprandial hyperglycemia, such as acarbose, miglitol and voglibose are well known.[15] It has been demonstrated in an animal model of chronic BHV that glucosidase inhibitors can alter glycosylation and have anti-viral activity. As the mechanism of action of α-glucosidase inhibitors is the induction of misfold or otherwise defective glycoproteins such inhibitors may be

Table 1. Enzyme Selectivity [IC_{50} Values (μM)] of Compounds **1**—**4** for α-Glucosidase

Compound	α-Glucosidase IC_{50}[a] (μM)
1	65.5±2.5
2	Not active
3	757.8±65.5
4	577.7±19.0
Deoxynojirimycin	425.6±8.1

a) IC_{50} values are the mean±standard error mean (S.E.M.) of three assays.

useful therapeutics for many viruses, especially those which bud from the endoplasmic reticulum (where protein folding takes place). For example bovine viral diarrhea virus, a pestivirus akin to hepatitis C virus, is also extremely sensitive to glucosidase inhibition.[16] α-Glucosidase inhibitors, such as DNJ (deoxynojirimycin), NB-DNJ, are potent inhibitors of human immunodeficiency virus (HIV) replication and HIV-mediated syncytium formation *in vitro*.[17] Inhibition of α-glucosidases causes abnormal functionality of glycoproteins because of incomplete modification of glycans. Suppressions of this processing is to be expected for antiviral activity and decreasing of growth rate of the tumor.[18] Compounds **1**—**4** were subjected to this assay and the results are listed in Table 1. The flavonoid **1** was more active than standard while **3** and **4** showed significant activity. The compound **2** was surprisingly non active from which it can be concluded that position of substituents in the aromatic rings of this class of flavonoids plays a vital role in enzyme inhibiting activity.

Experimental

General Column chromatography (CC): silica gel 70—230 mesh. Flash chromatography (FC): silica gel 230—400 mesh. TLC: pre-coated silica gel 60 F_{254} plates. UV: detection at 254 nm and by ceric sulphate reagent. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR spectra: Hitachi-UV-3200 and Jasco-302-A spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR, COSY, NOE, HMQC and HMBC spectra: Bruker spectrometers operating at 500, 400, 100 MHz; chemical shifts δ in ppm relative to $SiMe_4$ as internal standard and coupling constants J in Hz. EI-, FAB-, HR-EI-, HR-FAB-MS: JEOL JMS-HX-110 and JMS-DA-500 mass spectrometers, *m/z* (rel. int). The gas chromatography was performed on a Shimadzu gas chromatograph (GC-9A) (3% OV-1 silanized chromosorb W, column temperature 180 °C, injection port and detector temperature 275—300 °C, flow rate 35 ml/min, flame-ionization detector).

Plant Material The whole plant of *Duranta repens* LINN. (Verbenaceae) was collected from District Chitral N.W.F.P. (Pakistan), in April, 1997. The identity of the plant was verified by Prof. M. Qaiser, Department of Botany, University of Karachi where a voucher specimen (No. 52070) was deposited.

Extraction and Isolation The whole plant (20 kg) were shade dried, ground, and extracted with MeOH. The extract was suspended in water and extracted with $CHCl_3$ to yield a $CHCl_3$ fraction which was then subjected to medium pressure liquid chromatography (MPLC) on silica gel eluting with *n*-hexane–chloroform in increasing order of polarity to obtain six fractions. The fraction obtained from chloroform–methanol (9.8 : 0.2) was subjected to column chromatography over silica gel using *n*-hexane–ethylacetate in increasing order of polarity to obtain two sub-fractions A and B. The fraction A which eluted from *n*-hexane–ethylacetate (6 : 4) was a mixture of three major components. It was subjected to flash CC over silica gel using *n*-hexane–ethylacetate (4 : 6) as eluent to afford compound **5** (20 mg), **6** (24 mg) and **4** (18 mg), respectively. The fraction B which eluted from *n*-hexane–ethylacetate (4 : 6) was also subjected to flash CC over silica gel using *n*-hexane–ethylacetate (2 : 8) as eluent resulting in twenty sub-fractions. Fractions 1—10 which contained one major spot were purified through preparative TLC developing with chloroform–methanol (9.4 : 0.6) to yield compound **3** (12 mg). The fractions 10—20 were a mixture of two components which were resolved through preparative TLC developing with chloroform–methanol (9.2 : 0.8) to provide compound **1** (14 mg) and **2** (16 mg).

7-*O*-α-D-Glucopyranosyl-3,5-dihydroxy-3′-(4″-acetoxyl-3″-methylbutyl)-6,4′-dimethoxyflavone (**1**): Yellowish gummy solid. ^{1}H-NMR (CD_3OD, 400 MHz) in text. ^{13}C-NMR (CD_3OD, 100 MHz) δ: 180.0 (s, C-4), 175.2 (s, C-6″), 159.2 (s, C-4′), 156.1 (s, C-7), 154.2 (s, C-5), 153.6 (s, C-9), 153.2 (s, C-2), 138.4 (s, C-3), 131.1 (s, C-3′), 130.6 (s, C-6), 130.2 (d, C-2′), 128.3 (d, C-6′), 122.0 (s, C-1′), 116.1 (d, C-5′), 106.5 (s, C-10), 99.9 (d, C-1‴), 94.5 (d, C-8), 74.1 (d, C-5‴), 73.6 (d, C-3‴), 73.1 (d, C-2‴), 71.1 (d, C-4‴), 68.5 (t, C-4″), 62.8 (t, C-6‴), 60.1 (q, OCH_3-6), 55.6 (q, OCH_3-4′), 35.0 (d, C-3″), 34.2 (t, C-2″), 28.0 (t, C-1″), 23.2 (q, C-7″), 17.2 (q, C-5″). IR ν_{max} (KBr) cm^{-1}: 3396, 2925, 1656, 1597, 1190. UV λ_{max} (MeOH) nm (log ε): 273 (4.63), 343 (4.69). EI-MS *m/z*: 416 (72), 410 (32), 398 (22), 344 (12), 343 (8), 221 (6), 182 (4), 139 (10), 138 (7). FAB-MS *m/z* 621 $[M+H]^+$. HR-FAB-MS *m/z*: 621.2174 $[M+H]^+$ (calcd for $C_{30}H_{37}O_{14}$: 621.2183). $[\alpha]_D^{24}$ +27° (*c*=0.1, MeOH).

7-*O*-α-D-Glucopyranosyl-3,4′-dihydroxy-3′-(4″-acetoxyl-3″-methylbutyl)-5,6-dimethoxy-flavone (**2**): Yellowish gummy solid. ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.87 (1H, d, *J*=1.9 Hz, H-2′), 7.78 (1H, dd, *J*=8.5, 1.9 Hz, H-6′), 6.89 (1H, d, *J*=8.5 Hz, H-5′), 6.45 (1H, s, H-8), 5.30 (1H, d, *J*=3.7 Hz, H-1‴), 3.99 (1H, dd, *J*=9.5, 3.7 Hz, H-2‴), 3.86 (1H, t, *J*=9.5 Hz, H-3‴), 3.81 (3H, s, OCH_3-5), 3.76 (3H, s, OCH_3-6), 3.75 (1H, dd, *J*=11.4, 5.4 Hz, H-6‴b), 3.68 (1H, m, H-5‴), 3.64 (1H, dd, *J*=11.4, 4.8 Hz, H-6‴a), 3.56 (1H, t, *J*=9.5 Hz, H-4‴), 3.49 (2H, d, *J*=6.5 Hz, H-4″), 2.66 (2H, t, *J*=7.2 Hz, H-1″), 1.66 (1H, m, H-3″), 1.44 (2H, m, H-2″), 1.90 (3H, s, CH_3-7″), 0.99 (3H, d, *J*=6.6 Hz, CH_3-5″). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 180.0 (s, C-4), 175.3 (s, C-6″), 158.9 (s, C-4′), 158.5 (s, C-5), 157.4 (s, C-7), 153.6 (s, C-9), 153.3 (s, C-2), 138.6 (s, C-3), 132.1 (s, C-6), 131.4 (s, C-3′), 131.1 (d, C-2′), 128.5 (d, C-6′), 122.0 (s, C-1′), 116.2 (d, C-5′), 106.6 (s, C-10), 99.8 (d, C-1‴), 94.6 (d, C-8), 74.1 (d, C-5‴), 73.8 (d, C-3‴), 73.2 (d, C-2‴), 71.2 (d, C-4‴), 68.9 (t, C-4″), 62.9 (t, C-6‴), 61.5 (s, OCH_3-5), 60.5 (s, OCH_3-6), 35.2 (d, C-3″), 34.1 (t, C-2″), 28.5 (t, C-1″), 23.5 (q, C-7″), 17.5 (q, C-5″). IR ν_{max} (KBr) cm^{-1}: 3390, 2920, 1660, 1595, 1192. UV λ_{max} (MeOH) nm (log ε): 272 (4.41), 340 (4.47). EI-MS *m/z*: 416 (74), 401 (30), 373 (22), 342 (6), 207 (8), 152 (12). FAB-MS *m/z*: 621 $[M+H]^+$. HR-FAB-MS *m/z*: 621.2175 $[M+H]^+$ (calcd for $C_{30}H_{37}O_{14}$ 621.2183). $[\alpha]_D^{24}$ +14.2° (*c*=0.07, MeOH).

3,7,4′-Trihydroxy-3′-(8″-acetoxyl-7″-methyloctyl)-5,6-dimethoxyflavone (**3**): Yellowish gummy solid. ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.88 (1H, d, *J*=1.9 Hz, H-2′), 7.79 (1H, dd, *J*=8.5, 1.9 Hz, H-6′), 6.86 (1H, d, *J*=8.5 Hz, H-5′), 6.50 (1H, s, H-8), 3.88 (3H, s, MeO-5), 3.78 (3H, s, MeO-6), 3.48 (2H, d, *J*=6.6 Hz, H-8″), 2.68 (2H, t, *J*=7.2 Hz, H-1″), 1.92 (3H, s, CH_3-11″), 1.70 (1H, m, H-7″), 1.60 (2H, m, H-6″), 1.54 (4H, m, H-4″ and H-5″), 1.48 (4H, m, H-2″ and H-3″), 1.01 (3H, d, *J*=6.7 Hz, CH_3-9″). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 180.0 (s, C-4), 174.9 (s, C-10″), 159.0 (s, C-4′), 158.8 (s, C-5), 157.9 (s, C-7), 153.9 (s, C-9), 153.8 (s, C-2), 139.2 (s, C-3), 132.2 (s, C-6), 131.6 (s, C-3′), 131.0 (d, C-2′), 128.9 (d, C-6′), 122.0 (s, C-1′), 116.5 (d, C-5′), 107.0 (s, C-10), 95.5 (d, C-8), 69.0 (t, C-8″), 61.5 (q, OCH_3-5), 60.7 (q, OCH_3-6), 36.9 (d, C-7″), 34.6 (t, C-6″), 33.4 (t, C-5″), 32.8 (t, C-4″), 32.2 (t, C-3″), 30.1 (t, C-2″), 28.8 (t, C-1″), 24.0 (q, C-11″), 18.1 (q, C-9″). IR λ_{max} (KBr) cm^{-1}: 3395, 2910, 1665, 1596, 1195. UV ν_{max} (MeOH) nm (log ε): 272 (4.60), 344 (4.70). EI-MS *m/z*: 472 (69), 344 (42), 263 (80), 196 (12), 182 (6), 152 (9). FAB-MS *m/z*: 515 $[M+H]^+$. HR-FAB-MS *m/z*: 515.2270 $[M+H]^+$ (calcd for $C_{28}H_{35}O_9$: 515.2281). $[\alpha]_D^{24}$ +11.8° (*c*=0.06, MeOH).

(−)-6β-Hydroxy-5β,8β,9β,10α-cleroda-3,13-dien-16,15-olid-18-oic acid (**4**): Colourless gummy solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.15 (1H, t, *J*=1.5 Hz, H-14), 6.84 (1H, dd, *J*=4.5, 3.0 Hz, H-3), 4.77 (2H, d, *J*=1.5 Hz, H-15), 3.63 (1H, dd, *J*=11.0, 4.8 Hz, H-6), 2.29 (1H, m, H-2b), 2.20 (1H, m, H-2a), 2.15 (1H, ddd, *J*=13.0, 4.6, 1.6 Hz, H-12b), 2.01 (1H, ddd, *J*=13.0, 4.6, 1.6 Hz, H-12a), 1.69 (1H, m, H-1b), 1.68 (1H, ddd, *J*=14.0, 13.0, 4.6 Hz, H-11b), 1.62 (1H, m, H-7a), 1.59 (1H, m, H-8), 1.52 (1H, ddd, *J*=14.0, 13.0, 4.6 Hz, H-11a), 1.50 (1H, m, H-1b), 1.43 (1H, m, H-7b), 1.36 (1H, d, *J*=13 Hz, H-10), 1.21 (3H, s, CH_3-19), 0.82 (3H, d, *J*=6.6 Hz, CH_3-17), 0.76 (3H, s, CH_3-20). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 174.3 (s, C-16), 173.7 (s, C-18), 143.9 (d, C-14), 142.7 (d, C-3), 140.8 (s, C-4), 134.9 (s, C-13), 74.4 (d, C-6), 70.2 (t, C-15), 45.6 (d, C-10), 44.7 (s, C-5), 38.9 (s, C-9), 36.1 (t, C-11), 35.7 (t, C-7), 33.9 (d, C-8), 27.3 (t, C-2), 18.8 (t, C-12), 17.4 (q, C-20), 17.2 (t, C-1), 16.6 (q, C-19), 15.6 (q, C-17). IR ν_{max} (KBr) cm^{-1}: 3336, 2955, 1750, 1685. UV λ_{max} ($CHCl_3$) nm: 212. EI-MS *m/z* (rel. int.): 348 (2), 330 (18), 312 (63), 297 (6), 260 (39), 219 (23), 201 (30), 173 (100), 163 (41), 139 (29), 121 (78), 98 (51). HR-EI-MS *m/z*: 348.1925 (calcd for $C_{20}H_{28}O_5$: 348.1936). $[\alpha]_D^{24}$ −75° (*c*=0.2, MeOH).

Acid Hydrolysis of Compounds 1 and 2 A separate solution of **1** and **2** (7 mg) in MeOH (5 ml) containing 1 N HCl (4 ml) was refluxed for 4 h, con-

centrated under reduced pressure and diluted with H_2O (8 ml). It was extracted with EtOAc and the residue recovered from the organic phase was found to be an inseperatable mixture of products. The aqueous phase was concentrated and D-glucose was identified by the sign of its optical rotation ($[\alpha]_D^{27}$ +52.5°). It was also confirmed by comparing the retention time of its TMS ether of the glycone with standard sample (retention time α-anomer 4.1 min and β-anomer 7.8 min).

Enzyme Inhibition Assay The inhibitory activity of the compounds has been determined against α-glucosidase (E.C. 3.2.1.20), from saccharomyces *sp.* purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). The inhibition has been measured spectrophotometrically at pH 6.9 and at 37 °C using 1 mM *p*-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate and 0.69 units/ml enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin (0.425 mM) was used as a positive control.[19] The increment in absorption at 400 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with the spectrophotometer (Molecular Devices U.S.A.).[14]

Estimation of IC_{50} Values The concentration of the test compounds which inhibited the hydrolysis of PNP-G by α-glucosidase by 50% (IC_{50}) were determined by monitoring the effect of increasing the concentration of these compounds in the assays of the inhibition values. The IC_{50} values were then calculated using EZ-Fit enzyme kinetics program (Perrella Scientific Inc., Amherst, MA, U.S.A.).

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