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PHOSPHODIESTERASE INHIBITORY COUMARINOLIGNOIDS FROM *DURANTA REPENS*

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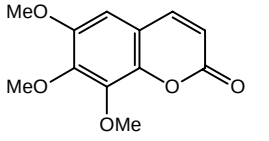
Abstract-Durantins A-C **(1-3)**, new coumarinolignoids together with three known compounds cleomiscosins A **(4)**, 6,7,8-trimethoxycoumarin **(5)** and 5,4′-dihydroxy-3,6,7-trimethoxyflavone **(6)** have been isolated from a $CHCl_3$ soluble fraction of *Duranta repens.* Their structures were assigned on the basis of spectral studies. Compounds **(1**, **3** and **4)** showed inhibitory activity against phosphodiesterase enzyme.

Duranta repens 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.[1]The methanolic extract also shows insecticidal and antifeedant properties.[2] A steroid,[3] diterpenoids and *C*-alkylated flavonoids have previously been reported from this species.[4] The ethanolic extract of this plant showed strong cytotoxicity in brine shrimp lethality test.[5] Further pharmacological screening of the ethanolic extract revealed inhibitory activity against phosphodiesterase enzyme.[6] The enzyme nucleotide pyrophosphatases/phosphodiesterases (NPP1) or plasma cell antigen 1 (PC-1) successively hydrolyses 5′-mononucleotides from nucleotides and their derivatives.[7] They exist both as membrane proteins with an extracellular active site and as soluble proteins in body fluid.[8] They are widely distributed in mammalian intestinal mucosa, mammalian liver cells, blood serum, snake venom and various plants.[6] NPPs are believed to be involved in a wide variety of processes, such as bone formation, insulin resistance and metastasis of cancer cells. Inhibitors of PC-1 might be useful as treatment for some forms of arthritis.[6,9,10] Herein we report the isolation and structural elucidation of durantins A-C **(1-3)**, three new coumarinolignoids along with the known compounds cleomiscosin A **(4)**, 6, 7, 8-trimethoxycoumarin **(5)** and 5, 4′ dihydroxy-3, 6, 7-trimethoxyflavone **(6)** reported for the first time from this species.[11-13] The compounds **(1**, **3** and **4)** showed inhibitory activity against phosphodisterase.

Durantin A (1) was isolated as a creamish yellow amorphous powder. The molecular formula was determined as $C_{22}H_{20}O_9$ by an $[M+H]^+$ peak at *m/z* 429.1190 in HR-FAB-MS spectrometry (calcd for

$C_{22}H_{21}O_9$, 429.1185). The UV maxima at 326 and 285 nm and the IR absorption at 1721 cm^{-1} suggested that **1** was a coumarin derivative. This was supported by the presence of two characteristic doublets of the coumarin methine protons H-3 at δ 6.31 and H-4 at δ 7.86 with a coupling constant of 9.5 Hz in the ^{1}H-NMR spectrum.[14] Additional features of **1** could be inferred from the chemical shifts and splitting pattern of its ^{1}H-NMR spectral signals including one phenolic proton at δ 8.55 (1H, s), three aromatic methines giving an ABX pattern in which hydrogen at δ 7.05 appeared as a doublet (*J* = 1.9 Hz), another hydrogen appeared as double doublet at δ 6.94 (*J* = 8.1, 1.9 Hz) while the remaining hydrogen at δ 6.85 gave a doublet (*J* = 8.1 Hz). It further showed two aromatic methoxyl groups at δ 3.86 and δ 3.87 (3H, s) and the propanoid moiety δ 5.04 (1H, d, *J* = 8.0 Hz), 4.18 (1H, ddd, *J* = 8.0, 2.8, 1.9 Hz), 3.53 (1H, dd, *J* = 12.6, 2.8 Hz) and 3.80 (1H, dd, *J* = 12.6, 1.9 Hz)] for H-7′, H-8′, H-9′b and H-9′a respectively.[15] The above spectral data were closely related to those of cleomiscosin A[11] **(4)** differing in having additional methyl singlet at δ 1.86. The fragmentation pattern in the EIMS spectrum was also very similar to cleomiscosin A[11] **(4)**, showing strong peak at *m/z* 386 due to the loss of C_2H_2O moiety. Further diagnostic fragments *a*, *b* and *c* at *m/z* 208, 180 and 137 originated from the ion at *m/z* 386 (link scan measurements) and confirmed the presence of one methoxyl group in the coumarin moiety and the remaining methoxyl and phenolic groups in the phenyl ring. The ^{13}C-NMR (BB, DEPT) spectra of **1** corroborated the presence of three methyl, one methylene, eight methine, and ten quaternary carbons. The signals at δ 176.2 and δ 23.2 were due to an acetyl moiety. Its presence at C-8′ and that of trisubstituted phenyl at C-7′ were illustrated through HMBC correlations (Figure 1). The coupling constant (*J* = 8.0 Hz) between the two vicinal oxymethine protons of C-7′ and C-8′ indicated that the phenyl group and the acetoxymethyl were *trans* diaxial.[16] The presence of methoxyl groups at C-6 and C-3′ and phenolic group at C-4′could also be inferred through HMBC correlations. The coumarinolignans occur naturally as regioisomeric pairs due to the linkage of the benzodioxan moiety to the coumarin core. The structure **(1)** was finally assigned by selective heteronuclear decoupling.[17] When the C-7′ and C-8′ hydrogen signals (δ 5.04 and 4.18) were irradiated, the carbon signals for C-7 (δ 137.1) and C-8 (δ 132.0) respectively showed significant sharpening.



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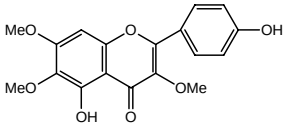

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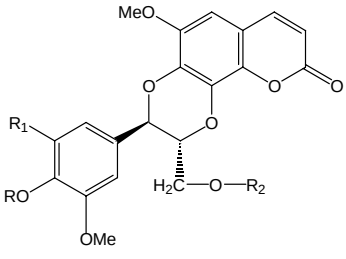
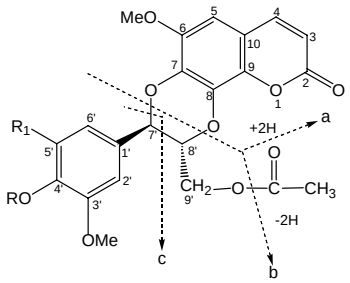


1, R = R_1 = H, R_2 = $COCH_3$
2, R = Me, R_1 = H, R_2 = $COCH_3$
3, R = H, R_1 = OMe, R_2 = $COCH_3$
4, R = R_1 = R_2 = H



1, R = R_1 = H
2, R = Me, R_1 = H
3, R = H, R_1 = OMe

Mass fragmentation pattern of compounds **(1-3)**

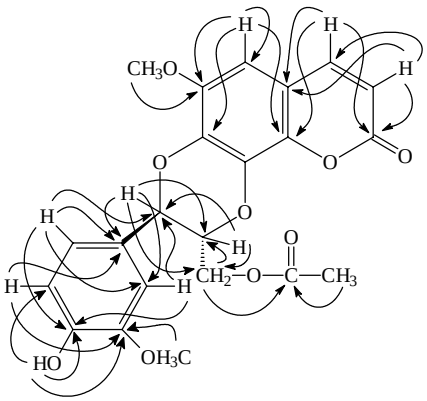


Figure 1 Important HMBC correlations of **1**

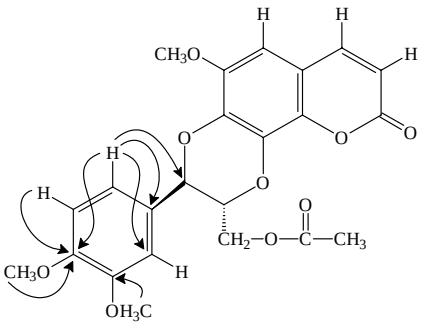


Figure 2 Important HMBC correlations of the phenyl ring of **2**

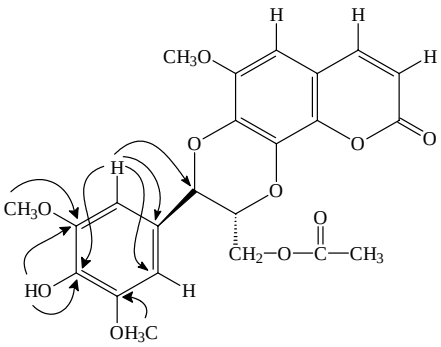


Figure 3 Important HMBC correlations of the phenyl ring of **3**

Durantin B (2) was obtained as a creamish yellow amorphous solid, molecular formula $C_{23}H_{22}O_9$ by $[M+H]^+$ peak at *m/z* 443.1345 in HR-FABMS spectrometry (calcd for $C_{23}H_{23}O_9$, 443.1341). The UV, IR, ^{1}H-NMR and MS spectra closely resembled to those of **1**. The difference was an additional signal of methoxyl group instead of phenolic. It was confirmed by the ^{1}H-NMR spectrum of **2** in which an additional signal at δ 3.90 was observed. The MS fragmentation pattern differed from **1** in having fragments *b* and *c* at *m/z* 194 and 151 which established that the two methoxyls were present in the phenyl propanoid moiety.[11] The structure was further confirmed by the HMBC spectrum (Figure 2). The methoxyl group at δ 3.90 was correlated with C-4′ (δ 149.8). The H-6′ at δ 6.94 showed interactions with C-4′ (δ 149.8), C-2′ (δ 111.8) and C-7′ (δ 77.1) further confirming the position of the methoxyl group at C-4′. Consequently, the spectral data of durantin B were in complete agreement to the assigned structure **(2)**.

Durantin C (3) was isolated as a creamish yellow amorphous powder. The molecular formula was shown to be $C_{23}H_{22}O_{10}$ by $[M+H]^+$ peak at *m/z* 459.1298 in HR-FABMS (calcd for $C_{23}H_{23}O_{10}$, 459.1291). The UV, IR, MS, and ^{1}H-NMR spectra of compound **(3)** resembled to those of **1**. The EIMS spectrum gave fragments *a, b* and *c* at *m/z* 208, 210 and 167 indicating the presence of two methoxyls and one phenolic functionalities in the phenyl propanoid moiety and one methoxyl group in the coumarin nucleus.[18-19] The only difference in the ^{1}H-NMR spectrum was the substitution pattern of phenyl ring in which two aromatic methines at δ 6.77 (2H, s) and two aryl methoxyls at δ 3.88 (6H, s) were shown to be chemically equivalent confirming the symmetrical substitution pattern of the aromatic ring.[14] These assignments were further confirmed by HMBC correlations (Figure 3) which were in accordance to the structure **(3)**.

It is interesting to note that the compounds **(1-3)** showed no optical activity and are therefore racemic.[14,17] The compounds **(1-6)** were tested for their inhibiting activity against snake venom phosphodiesterase 1 using cystein and EDTA as positive controls. Compounds **(1, 3** and **4**[11]**)** showed

moderate to strong inhibitory activity against phosphodiesterase while **2** showed weak activity and **5**[12], **6**[13] were inactive against this enzyme. Usually papaverine (IC_{50} *ca*. 30 μM) is applied as positive control but it could not be used due to inactivity against snake venom phosphodiesterase 1.[20] Nevertheless the compounds **(1-4)** can be regarded as weak inhibitors compared to papaverine.

Table 1 IC_{50} values of compounds **(1-6)** against phosphodiesterase**.**

Compound	IC_{50} with SEM
Durantin A (**1**)	184 μM ± 0.0027
Durantin B (**2**)	2750μM ±0.008
Durantin C (**3**)	884 μM ± 0.003
Cleomiscosin A (**4**)	559 μM 0.005
Compound (**5**)	- ve
Compound (**6**)	- ve
Cystein	748 μM ± 0.015
EDTA	274 μM ± 0.007

EXPERIMENTAL

General: Optical rotations were measured with a JASCO DIP-360 digital polarimeter. IR spectral data were taken on a JASCO 302-A spectrophotometer. UV spectra were obtained on a Hitachi UV-3200 spectrophotometer. The NMR spectra were run on Bruker spectrometers (^{1}H: 400 and 500 MHz; ^{13}C:100 MHz) in $CDCl_3$ and CD_3OD solutions, using SiMe4 as internal standard. EI, FAB and FAB-HRMS spectra were recorded on a Jeol, JMX-HX-110 and JMS-DA-500 mass spectrometer. Silica gel 60, 70-230 mesh and 200-440 mesh (both from E. Merck) were used for column and flash chromatography, respectively. Silica gel plates (Si 60 F254, E. Merck) were used for TLC.

Plant Material: The whole plant of *Duranta repens* (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. A voucher specimen (No. 52070) deposited in the herbarium of the University of Karachi, Pakistan.

Extraction and isolation: The ground plant (20 kg) was extracted with EtOH (50 L) at room temperature for 15 days yielding 600 g of a dark greenish extract. The extract was suspended in water (500 mL) and extracted with $CHCl_3$ (5 L) to yield a $CHCl_3$ fraction (285 g) which was then subjected to medium pressure liquid chromatography on silica gel eluting with *n*-hexane, *n*-hexane-$CHCl_3$, $CHCl_3$ and $CHCl_3$-MeOH in increasing order of polarity to obtain six fractions (A-F). The fraction B obtained from medium pressure liquid chromatography (*n*-hexane-$CHCl_3$ 6:4) was further subjected to vacuum liquid chromatography with a solvent gradient from *n*-hexane-EtOAc to afford three fractions. The fraction obtained from *n*-hexane-EtOAc (9:1) was further purified by column chromatography using silica gel (70-230 mesh) eluting with *n*-hexane-EtOAc (8:2) to furnish compound **(5)**[12] (7 mg) and **(6)**[13] (9 mg). The fraction obtained from *n*-hexane-EtOAc (6:4) was rechromatographed over flash silica using *n*-

hexane-EtOAc (6:4-2:8) to give two successive major fractions The first fraction was a mixture of two compounds which were separated by preparative TLC using solvent system n-hexane-EtOAc (1:1) to yield **(1)** (15 mg) and **(4)**[11] (12 mg). The second fraction was also a mixture of two compounds which were separated by preparative TLC using *n*-hexane-EtOAc (4:6) to furnish **(2)** (8 mg) and **(3)** (10 mg), respectively.

The compounds **(1-6**) were obtained as minor constituents. The major compounds were C-alkylated flavonoids, diterpenes and steroid which were isolated from fractions C-F. These have been previously reported from the same species[3,4] and are not described in this communication.

Enzyme Inhibitory Assay: Activity against snake venom phosphodiesterase 1 (Sigma P 4631) (EC 3.1.4.1) was assayed by using the reported method[20] with the following modification: 33 mM *Tris*- HCl buffer pH 8.8, 30 mM Mg-acetate with 0.000742 U/well final concentration using microtiter plate assay and 0.33 mM *bis*- (*p*-nitrophenyl) phosphate (Sigma N - 3002) as a substrate. From Merck cystein and EDTA[7,21-23] were used as positive controls (IC_{50} = 748 μM ± 0.015, 274 μM ± 0.007 respectively). After 30 min incubation the enzyme activity was monitored spectrophotometrically at 37 ^{o}C on a microtitre plate reader (Spectra Max, Molecular Device) by following the release of *p*-nitrophenol from *p*-nitrophenyl phosphate at 410 nm. Assay was conducted in triplicate.

Table 1 ^{13}C-NMR chemical shifts of compounds **(1-3).**

Position	**1**[a]	**2**[b]	**3**[b]
2	160.1 s	160.3 s	160.4 s
3	114.2 d	113.9 d	113.8 d
4	144.0 d	144.1 d	144.3 d
5	100.7 d	100.8 d	100.9 d
6	145.7 s	146.0 s	145.8 s
7	137.1 s	137.3 s	137.5 s
8	132.0 s	131.7 s	131.9 s
9	140.1 s	140.0 s	139.6 s
10	111.9 s	111.8 s	111.5 s
1′	130.9 s	130.3 s	130.5 s
2′	111.9 d	111.8 s	105.9 d
3′	151.1 s	150.0 s	150.0 s
4′	148.6 s	149.8 s	139.6 s
5′	116.3 d	116.2 d	150.0 s
6′	121.8 d	121.7 d	105.9 s
7′	77.3 d	77.1 d	77.0 d
8′	76.6 d	76.4 d	76.5 d
9′	62.5 t	62.3 t	62.0 t
O<u>C</u>OCH$_3$	176.2 s	176.1 s	176.0 s
OCO<u>C</u>H$_3$	23.2 q	23.1 q	23.0 q
MeO-6	56.4 q	56.4 q	56.3 q
MeO-3′	56.8 q	56.7 q	56.8 q
MeO-4′	-	56.8 q	-
MeO-5′	-	-	56.8 q

a measured in $CDCl_3$; b measured in $CDCl_3$+CD_3OD

Durantin A (1): Creamish yellow amorphous powder; $[\alpha]_D^{25}$ ±0^{o} (*c* 0.02, MeOH); UV (MeOH) λ_{max}

(log ε): 285 (3.62), 326 (4.09) nm; IR (KBr) ν_{max}: 3430, 2934, 1721, 1630, 1591 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.86 (3H, s, OCOC<u>H</u>$_3$), 3.53 (1H, dd, *J* = 12.6, 2.8 Hz, H-9′b), 3.80 (IH, dd, *J* = 12.6, 1.9 Hz, H-9′a), 3.86 (3H, s, OCH_3-6), 3.87 (3H, s, OCH_3-3′), 4.18 (1H, ddd, *J* = 8.0, 2.8, 1.9 Hz, H-8′), 5.04 (1H, d, *J* = 8.0 Hz, H-7′), 6.31 (1H, d, *J* = 9.5 Hz, H-3), 6.82 (1H, s, H-5), 6.85 (1H, d, *J* = 8.1 Hz, H-5′), 6.94 (1H, dd, *J* = 8.1, 1.9 Hz, H-6′), 7.05 (1H, d, *J* = 1.9 Hz, H-2′), 7.86 (1H, d, *J* = 9.5 Hz, H-4), 8.55 (1H, s, OH-4′); ^{13}C-NMR spectral data are shown in **Table 1**; EIMS *m/z* (rel. int.): 386 (69) $[M-42]^+$, 208 (46.1), 180 (69.57), 137 (100); FABMS *m/z* 429 $[M+H]^+$; HR-FABMS *m/z* 429.1190 $[M+H]^+$ ($C_{22}H_{21}O_9$ requires 429.1185).

Durantin B (2): Creamish yellow amorphous powder; $[\alpha]_D^{25}$: ±0° (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε): 289 (3.78), 329 (4.03) nm; IR (KBr) ν_{max}: 3432, 2935, 1720, 1629, 1590 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$: MeOH) δ: 1.87 (3H, s, OCOC<u>H</u>$_3$), 3.54 (1H, dd, *J* = 12.6, 2.6 Hz, H-9′b), 3.83 (1H, dd, *J* = 12.6, 1.8 Hz, H-9′a), 3.85 (3H, s, OCH_3-6), 3.88 (3H, s, OCH_3-3′), 3.90 (3H, s, OCH_3-4′), 4.18 (1H, ddd, *J* = (8.1, 2.6, 1.8 Hz, H-8′), 5.03 (1H, d, *J* = 8.1 Hz, H-7′), 6.30 (1H, d, *J* = 9.5 Hz, H-3), 6.80 (1H, s, H-5), 6.84 (1H, d, *J* = 8.1 Hz, H-5′), 6.94 (1H, dd, *J* = 8.1, 1.9 Hz, H-6′), 7.04 (1H, d, *J* = 1.8 Hz, H-2′), 7.85 (1H, d, *J* = 9.5 Hz, H-4); ^{13}C-NMR spectral data are shown in **Table 1**; EIMS *m/z* (rel. int.): 386 (70) $[M-42]^+$ 208 (44.4), 194 (65), 151 (100); FABMS *m/z* 443 $[M+H]^+$; HR-FABMS *m/z* 443.1345 $[M+H]^+$ ($C_{23}H_{23}O_9$ requires 443.1341).

Durantin C (3): Creamish yellow amorphous powder; $[\alpha]_D^{25}$: ±0° (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε): 233 (3.28), 323 (4.10) nm; IR (KBr) ν_{max}: 3433, 1721, 1628, 1585 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$: MeOH) δ: 1.87 (3H, s, OCOC<u>H</u>$_3$), 3.54 (1H, dd, *J* = 12.5, 2.7 Hz, H-9′b), 3.82 (1H, dd, *J* = 12.5, 1.9 Hz, H-9′a), 3.87 (3H, s, OCH_3-6), 3.88 (6H, s, OCH_3-3′ and 5′), 4.19 (1H, ddd, *J* = 8.1, 2.7, 1.9 Hz, H-8′), 5.04 (1H, d, *J* = 8.1 Hz, H-7′), 6.32 (1H, d, *J* = 9.5 Hz, H-3), 6.77 (1H, s, H-2′ and H-6′), 6.82 (1H, s, H-5), 7.87 (1H, d, *J* = 9.5 Hz, H-4), 8.55 (1H, s, OH-4′); ^{13}C-NMR spectral data are shown in **Table 1**; EIMS *m/z* (rel. int.): 386 (73) $[M-42]^+$, 208 (44.8), 210 (53.6), 167 (100); FABMS *m/z* 459 $[M+H]^+$; HR-FABMS *m/z* 459.1298 $[M+H]^+$ ($C_{23}H_{23}O_{10}$ requires 459.1298).

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