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FLAVANOCOUMARINS AND FLAVANOPHENYLPROPANOIDS FROM *PHYLLOCLADUS TRICHOMANOIDES*

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Key Word Index—*Phyllocladus trichomanoides*; Podacarpaceae; flavanocoumarins; flavanophenylpropanoids; structure elucidation.

Abstract—Two novel flavanocoumarins, epiphyllocoumarin and phyllocoumarin together with two flavanophenylpropanoids epicatechin-[5,6-*e*]-4β-(3′,4′-dihydroxyphenyl)-dihydro-2(3H)-pyranone and the novel 3-*O*-[β-hydroxy-δ-(3,4-dihydroxyphenyl)-pentanoyl]-catechin-[5,6-*e*]-4β-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranone or isophylloflavanine were isolated from the ethyl acetate extract of the cladodes of *Phyllocladus trichomanoides*. The structures of these compounds were established on the basis of their ^{1}H and ^{13}C NMR data.

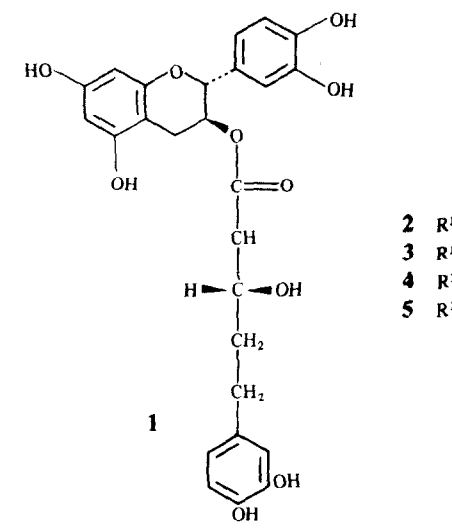
INTRODUCTION

Phyllocladus trichomanoides D. Don more commonly known as the Celery pine is an evergreen tree endemic to both the North and South Islands of New Zealand. To the Maori the tree is known as Tanekaha and various parts of the tree have been employed by them for treatment of various medical problems including dysentery, hepatitis, and skin disease [1]. In an earlier investigation of the cladodes of the tree the presence of phylloflavan, a major constituent possessing hypertensive properties [2] together with other major polyphenolics were reported [3]. In the course of their isolation the presence of a number of other components at low levels were noted and the present report deals with the isolation and structural elucidation of some of these minor constituents.

RESULTS AND DISCUSSION

Previous column chromatography [3] of the ethyl acetate extractives of the cladodes of *Phyllocladus trichomanoides* alternating between Sephadex LH 20 and MCl gel had afforded (+)-catechin, (−)-epicatechin, phylloflavan (**1**), cinchonian 1b (**2**), cinchonain 1a (**3**) the corresponding catechin analogues (**4** and **5**) and the phylloflavan analogue (**6**) together with other unresolved chromatographic fractions. These latter fractions were further processed on Sephadex LH 20 columns using dilute aqueous ethanol as solvents to yield two flavanocoumarins (**7** and **8**) and two phenylpropanoids (**9** and **10**).

Compound **7**, $[\alpha]_{589}$ −400° (MeOH; c 0.05) appeared under UV as a light blue fluorescent spot which intensified on exposure to ammonia vapour. The ^{13}C NMR spectrum of 7 was relatively simple showing evidence of a catechin core with chemical shifts at δ82.6, 67.0 and 28.5 which were associated with the C-2, C-3 and C-4 respectively of heterocyclic carbons of flavans with the 2,3-*trans* stereochemistry [4] as well as showing aromatic carbons resonances identifiable with the phloroglucinol A-ring and the catechol B-ring systems (see Table 1). However, only one unsubstituted A-ring carbon (δ95.6) was observable indicating the presence of a substituent in the ring, a deduction supported by the observation of a signal at δ105.8 attributable to that substituted carbon. The ^{1}H NMR spectrum of **7** also supported this structure, with resonances and couplings at δ4.88 (*d*, $J = 7.1$ Hz) and δ4.22 which were assigned to the H-2 and H-3 respectively of the catechin moiety and accompanied by resonances readily analysed for an ABX system characteristic of the catechol B-ring. Only one A-ring proton was observed as a singlet at δ6.41 which confirmed earlier indication from ^{13}C NMR data of the presence of a substituent in the A-ring. In addition to the flavan-3-ol resonances, two sets of doublets each accounting for one proton and coupled to each other ($J = 9.6$ Hz) were also observed in the low field region (δ6.10 and 7.97) of the proton spectrum. These resonances were consistent with the pyrone ring protons of a coumarin entity [5–7]. The coumarin structure was supported by IR data which showed a strong carbonyl absorption at 1690 cm^{-1} and further corroborated by the UV spectrum with maxima at 284 and 328 nm which shifted to longer wavelength on addition of base [5]. Positive ion FABMS of **7** gave an $[M+H]^+$ peak at *m/z* 343 which was also fully consistent with the proposed chemical constitution. The methine carbon resonances in the ^{13}C NMR spectrum of **7** observed at δ110.0 and 140.6 and the quaternary carbon resonance at δ160.9 could now be assigned to the α-, β- and carbonyl carbons respectively of the 2-pyranone ring. These values were fully consistent with published data for the respective carbon resonances in coumarins [7–9]. On biosynthetic considerations and on the basis of the structure of the co-occurring cinchonains [3, 10] two possible regioisomeric structures for the coumarin were likely, the first being where the 2-pyranone ring was formed between C-8 and the 7-OH while the second was between the C-6 and 5-OH of the flavan A-ring. In the cinchonains, the chemical shifts of the unsubstituted A-ring carbon and the C-4a carbon were diagnostic for locating the site of the pyranone ring. In **7** the chemical shift of the unsubstituted A-ring carbon (δ95.6) and the C-4a carbon (δ102.9) were comparable to those of the cinchonains



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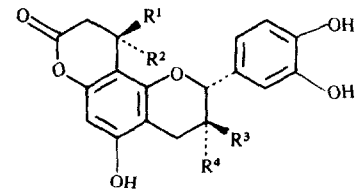
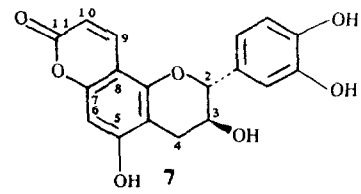


2 R^1 = -(3,4-dihydroxyphenyl), R^2 = R^3 = H, R^4 = OH
3 R^1 = R^3 = H, R^2 = -(3,4-dihydroxyphenyl), R^4 = OH
4 R^1 = -(3,4-dihydroxyphenyl), R^2 = R^4 = H, R^3 = OH
5 R^1 = R^4 = H, R^2 = -(3,4-dihydroxyphenyl), R^3 = OH



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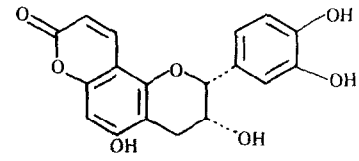

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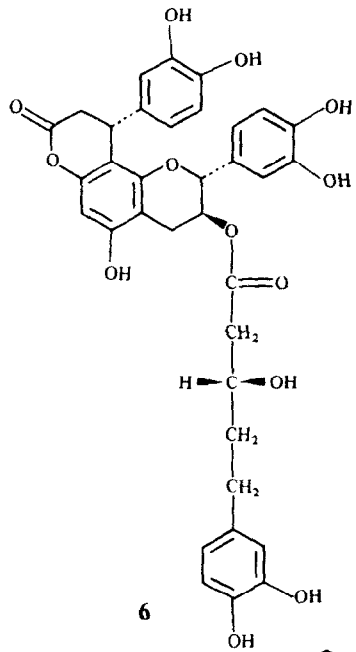

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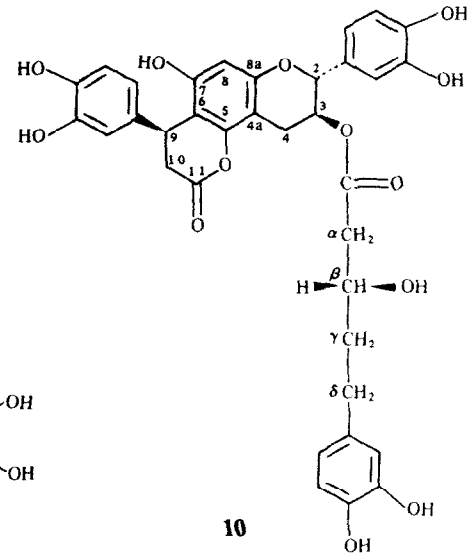

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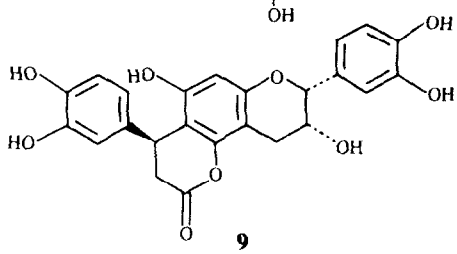

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with the pyranone ring formed between the C-8 and 7-OH and on this basis phyllo-coumarin was assigned the structure **7**. This chemical constitution was also supported by long-range coupling considerations, the so called chromen inter-ring couplings between protons separated by five bonds in an extended zig-zag pattern [11, 12]. Examination of the H-6 and H-9 resonances of the ^{1}H NMR spectrum of **7** showed the presence of such long-range couplings ($J = 0.4$ Hz) and only the structure of the coumarin where the H-6 and H-9 described such an extended zig-zag pattern was for the pyrone ring to be formed between the C-8 and the 7-OH.

The structure of **8**, $[\alpha] -100°$ (MeOH; c 0.04) followed readily from **7**. Like **7**, the FABMS of **8** also gave an $[M+H]^+$ at m/z 343 and a carbonyl frequency at 1689 cm^{-1} in the IR spectrum. The ^{1}H and ^{13}C NMR spectra were also very similar to those of **7**, the presence of the coumarin moiety was indicated by the proton chemical shifts observed at δ6.08 and 8.06 and substantiated by the magnitude of their coupling constants ($J = 9.6$ Hz). Simi-

Table 1. Distinctive ^{13}C NMR chemical shifts of flavano-coumarins and flavano-phenylpropanoid compounds in MeCO-d_6–H_2O (1:1 v/v)

		7	8	2	9	1	6	10
Flavan moiety	C-2	82.6	79.6	79.2	79.4	78.2	78.0	78.5
	C-3	67.0	65.7	66.3	66.1	69.8	69.3	69.7
	C-4	27.5	28.6	28.7	28.4	23.9	23.8	23.4
	C-4a	102.9	103.3	106.0	101.0	98.6	105.5	100.1
	C-6	95.6	95.7	96.9	106.8	96.5	96.9	107.1
	C-8	105.8	105.1	105.2	100.2	95.2	104.5	99.9
Pyranone moiety	C-9	140.6	141.1	34.2	34.4		33.9	34.3
	C-10	110.0	109.8	37.3	37.9		37.7	37.6
Pentanoyl moiety	αC					39.2	39.1	39.0
	βC					67.7	68.2	68.2
	γC					31.2	31.1	31.3
	δC					42.7	42.9	42.9

larly, the carbon resonances at δ109.8, 141.1 and 160.9 were fully consistent with α-, β- and the carbonyl carbons respectively of the 2-pyrone ring. The most significant difference between the ^{13}C NMR spectra of **7** and **8** was the upfield shift of the C-2 resonance (δ79.6) of the flavan-3-ol core consistent with the value observed for flavanoids with the 2,3-*cis* configuration [4]. This stereochemistry was also corroborated by the observation of a broad singlet (δ5.12) attributable to the H-2 in the ^1H NMR spectrum of **8**. The site of the pyrone moiety on the epicatechin A-ring was established by comparing the carbon chemical shift values of C-4a (δ103.3) and the unsubstituted A-ring carbon (δ95.7) with the cinchonains [3, 10] which suggested that the C-8 and the 7-OH of the A-ring was involved in the formation of the pyrone ring. Examination of the H-9 resonance revealed the presence of a small splitting ($J = 0.4$ Hz) of the doublet which was consistent with the type of inter-ring long-range coupling mentioned earlier. Hence the compound was the C-3 epimer of **7** and therefore named here as epiphyllocoumarin.

The acetate and the shikimate pathways are two major biosynthetic routes which give rise to benzenoid ring systems in plants. The phloroglucinol A-ring in the flavanoids is generally accepted as having been derived from the acetate route while the B-ring is derived from the shikimate. The latter route also accounts for the biosynthesis of the coumarins. The isolation of the coumarins **7** and **8** which shared the benzenoid ring with the flavanoid A-ring therefore poses an interesting biosynthetic problem. A plausible explanation for the origin of these flavanocoumarins is that they were derived from the corresponding more abundant co-occurring cinchonains by an α-β-elimination of the appending catechol unit in the pyranone ring thus making these compounds as rare examples of acetate derived coumarins.

Compound **9**, $[\alpha]_{589} -40°$ (MeOH; *c* 0.01) was clearly related to the cinchonains **2** and **3** as evidenced by the similarity of their ^{13}C NMR spectra. The presence of an epicatechin core was apparent from the presence of the chemical shifts at δ79.4, 66.1 and 28.4 identifiable with the C-2, C-3 and C-4 respectively of the flavanoid unit and complemented by the aromatic chemical shift associated with the phloroglucinol A-ring and the catechol B-ring systems. Additional resonances attributable to a methine carbon (δ34.4), a methylene carbon (δ37.9) and a carbonyl carbon (δ171.0) together with the presence of an additional catechol ring carbon resonances were clearly consistent with a cinchonain constitution. This structure was further supported by the observation of a strong carbonyl stretching frequency at 1747 cm^{-1} characteristic of a six-membered lactone ring with an unsaturation in the γ-δ position [13]. This constitution was further confirmed by FABMS which gave an $(M+H)^+$ ion peak at *m/z* 453. These data could only indicate that **9** was the C-6 substituted regioisomer of **2** or **3**, a deduction which was fully justified by carbon chemical shifts considerations. The downfield position of the unsubstituted A-ring carbon (δ100.2) compared to the corresponding carbons (δ96.9 and δ96.8) in **2** and **3** respectively in conjunction with the associated upfield position of the C-4a (δ101.0) compared to (δ106.0) in both instances were clearly consistent with the pyrone ring linking the C-6 and 5-OH of the flavan A-ring [10]. The C.D. of **9** had a positive couplet at 285 nm and negative couplet at 253 nm indicating the catechol substituent on the pyrone ring had the β conformation and compound **9** was therefore epicatechin-[6,5-*e*]-4β-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranone, identical to cinchonain Ic isolated from *Cinchona succirubra* [10].

The ^1H and ^{13}C NMR spectra of compound **10**, $[\alpha] -17°$ (MeOH; *c* 0.04) showed resonances characteristic of a flavanoid core, a δ-(3,4-dihydroxyphenyl)-β-hydroxypentanoyl side-chain [3] as in phylloflavan **1** and a pyranone entity as in the cinchonains (Table 1). Corroboration of this chemical constitution was available from FABMS which gave an $[M+H]^+$ ion peak at *m/z* 661 and the 2D ^1H NMR spectrum (COSY) of the compound was fully consistent with the structure. The downfield position of both the H-3 (δ5.38) and C-3 (δ69.7) resonances relative to the unsubstituted flavan-3-ol resonances clearly defined the location of the pentanoyl substituent as being at 3-OH on the flavan moiety. Thus **10** had to be an isomer of phylloflavanine **6**, a constituent of *Phyllocladus trichomanoides* isolated earlier [3]. Like phylloflavan and phylloflavanine both of which possessed the same bulky substituent at 0–3, the magnitude of the H-2 and H-3 coupling constants ($J = 5.6$ Hz) was indica-

tive of the 2,3-*trans* stereochemistry of the substituents on the heterocyclic flavanoid ring. The rather diminished J values were in full accord with suggestions of significant contribution of the *trans* di-axial conformation of the bulky C-2 and C-3 substituent hence keeping steric interaction between them to a minimum [14–16]. The location of the pyranone on the flavanoid A-ring could be made by similar considerations of the appropriate carbon chemical shifts as discussed earlier for the cinchonains. It was clear from Table 1 that the most significant differences between the carbon chemical shifts of **10** and phylloflavanine were limited to the unsubstituted A-ring carbon and C-4a, these resonances δ99.9 and 100.1 respectively were comparable to those in **9** and consistent with the pyranone ring being at C-6 in **10** as opposed to C-8 in phylloflavanine. The C.D. spectrum of (**10**) showed a weak positive couplet at 235 nm compared to phylloflavanine which had a strong negative couplet at 233 nm. The opposite direction of the c.d. couplet indicated the aromatic catechol substituent in the pyranone ring was above the plane of the A-ring [3, 10] and hence **10** was 3-*O*-[β-hydroxy-δ-(3,4-dihydroxyphenyl)-pentanoyl]catechin-[6,5-*e*]-4β-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranone or isophylloflavanine to conform with the common name proposed earlier.

The identification of **9** and **10** where the pyranone ring was attached to the C-6 in the flavanoid A-ring in addition to the more abundant C-8 substituted compounds isolated earlier [3] suggested that the reactivity of the A-ring was a dominant factor in determining the yields of the various regioisomers, a condition much analogous to that encountered in natural procyanidins with respect to the relative levels of the C-4/C-8 and C-4/C-6 linked dimers and oligomers [17, 18]. Introduction of the pentanoyl functionality at the C-3 hydroxyl, however, appeared to be confined to flavanoids with the 2,3-*trans* configuration although compounds with the 2,3-*cis* configuration are more dominant in composition in the ethyl acetate extract of the cladodes of *Phyllocladus trichomanoides*. Thus this confirmed earlier suggestions [3] that there was strict enzymic control during the esterification of the C-3 hydroxy group.

EXPERIMENTAL

Optical measurements were made in MeOH and IR spectra were obtained with KBr pellets. TLC was on Schleicher and Schull cellulose plates using HOAc–H_2O (3:47, solvent A) and *t*-BuOH–HOAc–H_2O (3:1:1 v/v, solvent B) as the developing solvents. A voucher specimen of *P. trichomanoides* was lodged in the herbarium, Botany Division, Christchurch, New Zealand (CHR 388 244).

Extraction and isolation. Freeze-dried cladodes of *Phyllocladus trichomanoides* were milled and extracted and the EtOAc extract fractionated on Sephadex LH20 and MC1 Gel as previously described [3]. The unresolved chromatographic fractions from this work were further processed on a column of Sephadex LH20 using aqueous EtOH (H_2O–EtOH 17:3→7:3) to yield the flavanocoumarins **7** and **8** and the flavanophenylpropanoid compounds **9** and **10**.

Phyllocoumarin (**7**) isolated as freeze-dried solid (19 mg), $[\alpha]_{589}-400°$ (MeOH; *c* 0.05), R_f 0.15 (A), 0.85 (B). Positive FABMS gave $[M+H]^+$ at *m/z* 343. UV λ_{max}^{MeOH} nm: 284 and 328; 292 (NaOMe) and 378 (NaOMe). IR ν_{max} cm^{-1}: 3403, 1690, 1620, 1450, 1373, 1285, 1240, 1140, 1113, 820 and 764. ^{13}C NMR (Me_2CO-d_6–H_2O): δ27.5, 67.0, 82.6,, 95.6, 102.9, 105.8, 110.0, 115.1, 116.4, 119.5, 130.8, 140.6, 145.8 (×2), 152.3, 155.4, 160.9 and 163.5. ^{1}H NMR (Me_2CO-d_6): δ0.28–0.33 (H-4 obscured by H_2O peak), 4.20 (1H, *m*, H-3), 4.88 (1H, *d*, J = 7.1 Hz, H-2), 6.05 (1H, *d*, J = 9.6 Hz, H-10), 6.41 (1H, *d*, J = 0.4 Hz, H-6), 6.79–6.93 (3H, *m*, B-ring protons), and 7.97 (1H, *dd*, J = 9.6 Hz and 0.4 Hz, H-9).

epi*Phyllocoumarin* (**8**) isolated as freeze-dried solid (9 mg), $[\alpha]_{589}-100°$ (MeOH; *c* 0.04), R_f 0.05 (A) and 0.75 (B). Positive FABMS gave $[M+H]^+$ at *m/z* 34.3. UV λ_{max}^{MeOH} nm: 288 and 334; 296 (NaOMe) and 384 (NaOMe). IR ν_{max} cm^{-1}: 3440, 1689, 1620, 1527, 1450, 1373, 1285, 1250, 1140, 1105, 820, 800 and 770. ^{13}C NMR (Me_2CO-d_6–H_2O): δ28.6, 65.7, 79.6, 95.7, 103.3, 105.1, 109.8, 115.0, 116.5, 119.3, 131.0, 141.1, 145.1 (×2), 152.5, 155.0, 160.9 and 164.5. ^{1}H NMR (Me_2CO-d_6): δ2.5–3.8 (H_2O peak obscuring H-4), 4.36 (H, *m*, H-3), 5.13 (H, *br s*, H-2), 6.27 (1H, *d*, J = 9.8 Hz, H-10), 6.41 (1H, *br s*, H-6), 6.8–7.13 (3H, *m*, B-ring protons) and 8.05 (1H, *dd*, J = 9.6 and 0.4 Hz, H-9).

epi*Catechin*-[5,6-*e*]-4β-(3,4-*dihydroxyphenyl*)-*dihydro*-2(3*H*)-pyranone (**9**) obtained as freeze-dried powder (30 mg), $[\alpha]_{589}-40°$ (MeOH; *c* 0.01), R_f 0.38 (A), C.D. (MeOH): −ve (253 nm) and +ve (285 nm). Positive ion FABMS gave $[M+H]^+$ peak at *m/z* 452. IR ν_{max} cm^{-1}: 3200–3500, 1747, 1634, 1610, 1523, 1449, 1283, 1245, 1120, 1060. ^{13}C NMR (Me_2CO-d_6–H_2O): δ28.4, 34.4, 37.9, 66.1, 79.4, 100.2, 101.0, 106.8, 115.3 (×2), 116.5, 116.9, 119.1, 119.6, 131.5, 134.6, 144.4, 145.1 (×2), 145.6, 151.6, 153.6, 155.4 and 171.0. ^{1}H NMR (Me_2CO-d_6): δ2.5–3.5 (H-4 and H-10 obscured by H_2O peak), 4.29 (1H, *m*, H-3), 4.51 (1H, *dd*, J = 5.2 and 2.6 Hz, H-9), 4.96 (1H, *br s*, H-2), 6.30 (1H, *s*, H-8), 6.5–7.1 (6H, *m*, B-ring protons).

3-O-[β-*hydroxy*-δ(3,4-*dihydroxyphenyl*)-*pentpanoyl*]-*Catechin*-[5,6-*c*]-4β-(3,4-*dihydroxyphenyl*)-*dihydro*-2(3H)-*pyranone* (**10**) obtained as freeze-dried powder (12 mg) $[\alpha]_{589}-17°$ (MeOH; *c* 0.04), R_f 0.24 (A), 0.90 (B), C.D. (MeOH) +ve (235 nm). Positive FABMS gave $(M+H)^+$ peak at *m/z* 661. IR ν_{max} cm^{-1}: 3200–3500, 1727, 1610, 1522, 1450, 1285, 1245, 1115, 1060. ^{13}C NMR (Me_2CO-d_6–H_2O): δ23.4, 34.3, 37.6, 39.0, 42.9, 68.2, 69.7, 78.5, 99.9, 100.1, 107.1, 114.6, 115.4, 116.7 (×4), 118.8, 119.1, 120.8, 130.2, 134.5, 134.8, 143.3, 144.4, 145.2, 145.7 (×3), 150.8, 154.3 (×2), 170.4 and 172.5. ^{1}H NMR (MeCO-d_6): 1.63 (2H, *m*, H-γ), 2.40 (2H, *d*, J = 6.5 Hz, H-α), 2.42–3.2 (6H, *m*, H-δ, H-4 and H-10), 3.91 (1H, *m*, H-β), 4.50 (1H, *dd*, J = 1.8 and 6.2 Hz, H-9), 5.09 (1H, *d*, J = 5.6 Hz, H-2), 5.32 (1H, *m*, H-3), 6.36 (1H, *s*, H-8), 6.62–6.90 (9H, *m*, aromatic B-ring protons).

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