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NEW ISOFLAVONOIDS RELATED TO KIEVITONE FROM *PHASEOLUS VULGARIS**

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; French bean; isoflavanone; isoflavone; phytoalexin.

Abstract—The new isoflavonoids 5,7,2′,4′-tetrahydroxy-8-(3,3-dimethylallyl)isoflavone (2,3-dehydrokievitone) and 7,2′,4′-trihydroxy-8-(3,3-dimethylallyl)isoflavanone (5-deoxykievitone) have been isolated from fungus-inoculated *Phaseolus vulgaris* pod tissue and from the inoculation droplets. A third isoflavonoid was tentatively identified as 1″,2″-dehydrocyclokievitone and appears to be a metabolite of the phytoalexin kievitone.

INTRODUCTION

Kievitone **2** is one of the major antifungal isoflavonoids produced by *Phaseolus vulgaris* L. in response to fungal infection [1]. In addition to kievitone, four other isoflavonoids with the 5,7-dihydroxylation pattern in ring A have been identified from *P. vulgaris* tissue following fungal inoculation and, like kievitone, none of these was detected in healthy tissue [2]. Of these other isoflavonoids, only licoisoflavone A [3] (also phaseoluteone [2]) **5** was found in relatively large amounts, while the other three, genistein, 2′-hydroxygenistein **3**, and dalbergioidin, were found in smaller quantities. The latter three may constitute the isoflavonoid intermediates in kievitone biosynthesis. This paper reports the identification of 2,3-dehydrokievitone **4** which may be part of an alternative pathway leading to kievitone, and the isolation of two new isoflavanones related to kievitone.

RESULTS AND DISCUSSION

Quantitatively the most abundant of the minor components in inoculated tissue was a new isoflavanone ($C_{20}H_{20}O_5$) **1** for which the trivial name 5-deoxykievitone is suggested. Large bathochromic shifts in the UV spectrum produced by NaOH and NaOAc are consistent with the behaviour of other 5-deoxyisoflavanones [4, 5] and no shifts were observed with $AlCl_3$. The ^1H NMR spectrum (in $(CD_3)_2CO$) showed signals for five aromatic protons and confirmed the absence of the hydroxyl at C-5. The lowest field proton (δ 7.63, *d*) was coupled to a doublet at δ 6.64 and these protons are assigned to C-5 and C-6 of ring A, respectively (compare the respective protons of 7-hydroxy-4′-methoxyisoflavanone at δ 7.75 and 6.60 [5]). The remaining three aromatic protons form an ABX system (at δ 6.95, 6.44 and 6.31) and are assigned to C-6′, C-3′, and C-5′ of ring B based on a comparison with the respective protons in the spectrum of kievitone (δ 6.94, 6.45, 6.33). The presence of a 3,3-dimethylallyl group was indicated by signals at δ 1.65 (3H), 1.77 (3H), 3.35 (2H), and 5.25 (1H). Signals for the 3 heterocyclic ring protons were observed at δ 4.11 (*dd*), 4.58 (*dd*), and 4.68 (*dd*) with coupling constants of 11.0 (C-2a–C-2b), 9.7 (C-2a–C-3), and 5.3 Hz (C-2b–C-3). The pattern of the heterocyclic ring protons was modified when $CDCl_3$ was used as solvent and approximate coupling constants were obtained (δ 5.01, *dd*, $J \sim 12$, 3 Hz, C-2a; 4.81, *dd*, $J \sim 12$, 4 Hz, C-2b; and 3.90, *t*, $J \sim 3.5$ Hz, C-3). The C-3 proton in the spectrum of kievitone in $(CD_3)_2CO$ appeared as a doublet of doublets at δ 4.23 (J = 10.7, 5.2 Hz) while the same proton in the spectrum of cyclokievitone **8** in $CDCl_3$ appeared as a triplet at δ 3.97 (J = 4.9 Hz). These observations indicate that there is a change in the preferred conformation of these molecules when the solvent is changed from acetone to chloroform. The three hydroxyls can be assigned to C-7, C-2′, and C-4′ based on the above ^1H NMR data and on biogenetic grounds [6], which define the attachment of the 3,3-dimethylallyl group at C-8 in ring A.

Fragment ions in the mass spectrum of 5-deoxykievitone are consistent with structure **1**. The ion at *m/e* 136 $[C_8H_8O_2]^+$ corresponds to a retro-Diels–Alder (RDA) fragmentation with charge retention on the dihydroxylated ring B [7] and is identical to the corresponding fragment ion in the spectrum of kievitone [8]. The fragment ion at *m/e* 205 $[C_{12}H_{13}O_3]^+$ corresponds to a RDA fragmentation with hydrogen transfer and charge retention on ring A and the ion at *m/e* 149 $[C_8H_5O_3]^+$ represents the loss of C_4H_8 from the fragment at *m/e* 205 (m* = 108.2). The corresponding fragments in the spectrum of kievitone are at *m/e* 221 and 165 due to the additional oxygen on ring A.

The second compound ($C_{20}H_{18}O_6$) is a new isoflavone which has structure **4** (2,3-dehydrokievitone). The mass spectrum of 2,3-dehydrokievitone shows abundant fragment ions at *m/e* 311 $[C_{17}H_{11}O_6]^+$ and 299 $[C_{16}H_{11}O_6]^+$ which arise from the molecular ion by fragmentation of the side chain (m* = 273.2 and 252.5). Fragment ions at the same *m/e* are present in the spectrum of licoisoflavone A **5** [2]; however, unlike the spectrum of licoisoflavone A, the spectrum of 2,3-dehydrokievitone does not show a RDA fragment corresponding to ring B at *m/e* 147. Instead, the ring B RDA fragment ion occurs at *m/e* 134 $[C_8H_6O_2]^+$ (identical to the corresponding

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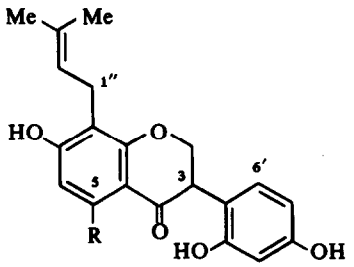
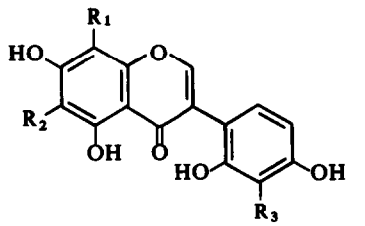
fragment ion in the spectrum of 2′-hydroxygenistein **3** [9]) and indicates that ring B is dihydroxylated. The fragment ion at *m/e* 165 $[C_8H_5O_4]^+$ appears to correspond to a RDA fragmentation with charge retention on ring A and arises from the fragment ion at *m/e* 299 (m* = 91.1). A computer program which examines spectral relatedness based on the number of common fragment ions and the correlation coefficient of the relative intensities of the common fragments [10] showed that the new isoflavone was similar to licoisoflavone A, but indistinguishable from luteone **6** [11]. TLC comparisons between the new isoflavone and luteone on polyamide and cellulose indicated that the two compounds might be different and this was confirmed by TLC on Si gel in $CHCl_3$–*iso*-PrOH (9:1) R_f **4** 0.22, **6** 0.39 and by treatment of the new isoflavone and luteone with HCOOH. Cyclization of luteone gave two isomers [11], but the new isoflavone gave only one isomer **9**. Bathochromic shifts induced in the UV spectrum of the new isoflavone **4** by NaOH, $AlCl_3$ and NaOAc indicated the presence of hydroxyls at C-5 and C-7 [12]. Hence, the 3,3-dimethylallyl group must be located at C-8. Ring A is thus unsubstituted at C-6 while the substitution pattern of ring B remained to be determined. The ^{1}H NMR spectrum of the cyclized isoflavone **9** in $(CD_3)_2CO$ showed signals for the C-5 OH at δ 12.54 and the isoflavone C-2 at 8.28 [13] (compare the respective protons of 2′-hydroxygenistein **3** at δ 12.79, 8.19). Three of the four aromatic protons appeared as an ABX system at δ 7.14 (*d*), 6.50 (*d*), and 6.45 (*dd*) and are assigned to C-6′, C-3′, and C-5′, respectively, based on the comparison with 2′-hydroxygenistein (δ 7.13, 6.49, 6.44).

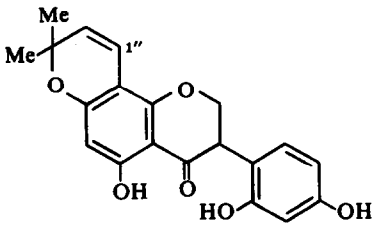
The third compound is a new isoflavanone ($C_{20}H_{18}O_6$) for which structure **7** is proposed. A bathochromic shift in the UV spectrum was induced by $AlCl_3$ indicating the presence of a hydroxyl at C-5; however, no shift was observed with NaOAc indicating the absence of a free hydroxyl at C-7. The ^{1}H NMR spectrum showed a signal for the C-5 hydroxyl proton at δ 12.49 and signals for four aromatic protons. A singlet integrating for one proton at δ 5.89 can be assigned to ring A (compare the proton at C-6 in kievitone, δ 6.05, and the C-8 proton of cajanone, δ 5.95 [14]). The three remaining aromatic protons appeared as an ABX system at δ 6.94 (*d*), 6.46 (*d*), and 6.34 (*dd*) and these chemical shifts are virtually identical to those of the C-6′, C-3′, and C-5′ protons of ring B in the spectrum of kievitone **2** and 5-deoxykievitone **1** as described above. The three protons of the heterocyclic ring were observed at δ 4.69 (*t*, J = 10.9 Hz), 4.55 (*dd*, J = 10.9, 5.7 Hz), and 4.29 (*dd*, J = 10.9, 5.8 Hz) and these are assigned to C-2a, C-2b, and C-3 (compare the respective protons of kievitone at δ 4.62 (*t*, J = 10.7 Hz), 4.51 (*dd*, J = 10.8, 5.2 Hz), and 4.23 (*dd*, J = 10.7, 5.2 Hz). The presence of a 2,2-dimethyl-



1 R = H
2 R = OH



3 $R_1 = R_2 = R_3 = H$
4 $R_2 = R_3 = H$; $R_1 = CH_2CH{=}CMe_2$
5 $R_1 = R_2 = H$; $R_3 = CH_2CH{=}CMe_2$
6 $R_1 = R_3 = H$; $R_2 = CH_2CH{=}CMe_2$



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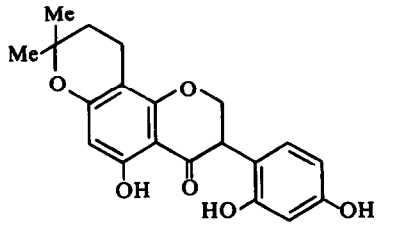

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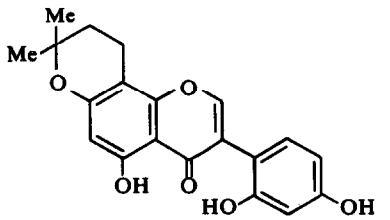

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chromene system in **7** is based on the appearance of two nearly equivalent methyl signals at δ 1.44 and 1.43 and two coupled olefinic protons (J = 10.0 Hz) at δ 6.54 and 5.63. In cajanone, the corresponding signals were observed at δ 1.42 (6H), 6.61 and 5.49 [14].

The fragment ion and base peak in the mass spectrum of **7** at *m/e* 339 $[M\text{-}Me]^+$ is characteristic of isoflavonoids with a 2,2-dimethylchromene system [7]. The ion at *m/e* 203 $[C_{11}H_7O_4]^+$ would be expected from a RDA fragmentation from the ion at *m/e* 339 with charge retention on ring A. A relatively low intensity ion at *m/e* 136 $[C_8H_8O_2]^+$ would arise from a RDA fragmentation with ring B carrying the charge. The other major fragment ion at *m/e* 321 results from the loss of H_2O from the fragment ion at *m/e* 339 (m* = 304.0).

The only point which remains to be clarified is whether the ring A proton occurs at C-6 or C-8. This question could not be resolved with the amount of material available; however, the biogenetic evidence strongly supports the attachment of the side chain at C-8 (1″,2″-dehydrocyclokievitone **7**) as the isoflavone **4**, and the isoflavanones **1** and **2** all have been isolated from *P. vulgaris* tissue and all have the side chain attached to C-8. No isoflavone (e.g. **6**) or isoflavanone with the 3,3-dimethylallyl side chain attached at C-6 has been isolated and this indicates that the enzyme which prenylates ring A does so specifically at C-8. The precursor of **7** would probably be kievitone **2** or cyclokievitone **8**; however, no evidence for the natural occurrence of cyclokievitone has been obtained. If structure **7** can be confirmed, this would indicate that kievitone metabolism occurs *in vivo* and considering that most of **7** was found in the inoculated tissue rather than the inoculation droplets (as was true for **1** and **4**), the enzyme responsible may be of host origin.

The occurrence of 2,3-dehydrokievitone **4** in *P. vulgaris* tissue extracts indicates the possible existence of two pathways leading to kievitone. One pathway would proceed from genistein → 2′-hydroxygenistein → dalbergioidin → kievitone with prenylation as the final step. The second possible pathway would diverge at 2′-hydroxygenistein **3** by prenylation to give 2,3-dehydrokievitone **4** which could then be reduced to give kievitone **2**. The relative significance of these possibilities in kievitone production is unknown.

The biosynthetic precursors of 5-deoxykievitone **1** are probably 5-deoxyisoflavonoids since the ring A oxidation pattern is established at the chalcone stage [15]. As *P. vulgaris* also produces large quantities of the pterocarpan phaseollin [16], 5-deoxykievitone and phaseollin may have common isoflavonoid precursors. Work is in progress to isolate the intermediates in phaseollin biosynthesis and to determine where the branch-point leading to 5-deoxykievitone occurs.

EXPERIMENTAL

All ^1H NMR spectra were obtained at 270 MHz with TMS as internal standard and δ values reported in this paper for reference compounds **2**, **3** and **8** were obtained in this study. Low resolution MS were obtained using a direct insertion probe (ionization voltage 70 eV; accelerating voltage 4 kV). Precision mass measurements were obtained using a direct insertion probe (ionization voltage 70 eV; accelerating voltage 8 kV, on-line computer). TLC solvent systems used were: I MeOH–H_2O (17:3), II Me_2CO–H_2O (99:1), III HOAc–H_2O (1:3), IV petrol(55–65°)–Et_2O–HOAc (25:75:1), V $CHCl_3$–MeOH (11:1), VI petrol(55–65°)–C_6H_6–EtOAc–MeOH (3:6:4:1). Systems I and II were used with polyamide [17], III with cellulose, and IV–VI with Si gel. Detection was by spraying with Fast Blue Salt B [17] for systems I–III and by I_2 for systems IV–VI.

Isolation of compounds **1**, **4**, *and* **7** *from inoculation droplets.* Pod cavities of *P. vulgaris* cv Red Kidney were prepared and inoculated with a conidial suspension of *Monilinia fructicola* (Wint.) Honey [18]. The inoculation droplets (9.5 l.) were collected after a 20 hr incubation period and lyophilized. The residue was dissolved in MeOH (2 l.), stored at −20° for several days, filtered, and taken to dryness *in vacuo*. The residue was suspended in 500 ml H_2O and partitioned against equal vols. of petrol (55–65°) (6×). The aq. layer was further partitioned against equal vols. of EtOAc (3×) and then discarded. The organic fractions were each taken to dryness under red. pres. and the EtOAc fraction was redissolved in 9.5 ml MeOH and 0.5 ml removed. The larger portion was reconcentrated and chromatographed on a column of perlon-type polyamide eluted with 85% EtOH. The eluate was divided into 5 fractions which contained: (A) phaseollin and phaseollidin, (B) phaseollidin and kievitone, (C) licoisoflavone A, (D) licoisoflavone A, dalbergioidin, and 2′-hydroxygenistein, and (E) 2′-hydroxygenistein as the major component(s). Subsequent separation of fraction B on a column of polyamide eluted with 85% MeOH gave several major phenolic components. 5-Deoxykievitone eluted just ahead of kievitone and was further purified on a column of Si gel eluted with a 0–3% gradient of MeOH in $CHCl_3$ followed by a column of LH-20 eluted with 95% EtOH. 2,3-Dehydrokievitone was found on the tail of the kievitone-containing fraction. After chromatography of fraction C on a column of polyamide eluted with 85% MeOH, additional 2,3-dehydrokievitone was obtained which eluted just prior to licoisoflavone A. Purification of the two crude fractions containing 2,3-dehydrokievitone was performed on individual columns of Si gel eluted with a 0–3% gradient of MeOH in $CHCl_3$. The partially purified compound was eluted with 2% MeOH and then chromatographed on a column of LH-20 eluted with 95% EtOH to give 2,3-dehydrokievitone. Compound **7** was not detected in the EtOAc fraction and an examination of the petrol fraction showed a small amount. Compound **7** was purified by chromatography on a column of polyamide eluted with 70% MeOH followed by a column of LH-20 eluted with 95% EtOH. Quantities of compounds **1**, **4**, and **7** isolated were 3.9, 6.4, and 0.43 μmol/9 l., respectively, as based on UV extinction coefficients for similar compounds (log ε in parentheses)—**4** is based on **6** (4.45) [11], **1** is based on 7,2′-dihydroxy-4′-methoxyisoflavanone (4.23) [19], and **7** is based on cajanone (4.30) [14].

Purification of **1**, **4** *and* **7** *from extracts of inoculated tissue.* The *P. vulgaris* endocarp tissue (3.75 kg) under the inoculation droplets was removed, placed in chilled MeOH (11 l.), and processed and extracted with petrol as described previously [2]. The petrol fraction was dissolved in 20 ml 90% MeOH and partitioned 3× against 20 ml petrol and the petrol was discarded. Chromatography of the methanolic soln on a column of polyamide eluted with 85% MeOH gave 5 fractions which contained predominantly (A) phaseollin and phaseollidin, (B) 5-deoxykievitone 1, (C) kievitone, (D) kievitone and licoisoflavone A, and (E) licoisoflavone A. Compound **7** was obtained from fraction A by chromatography on a column of polyamide eluted with 70% MeOH, followed by a column of Si gel eluted with a 0–3% gradient of MeOH in $CHCl_3$ and finally a column of LH-20 eluted with 95% EtOH. Compound **1** was further purified in the same manner. 2,3-Dehydrokievitone was isolated from fraction D after repeated chromatography on columns of polyamide

eluted with 70% and 85% MeOH followed by chromatography on a column of LH-20 eluted with 95% EtOH. Based on published extinction coefficients of the corresponding or related compounds (as listed above), amounts (μmol/3.75 kg fr. wt) obtained were: **1** 1.1 [19], **2** 39 [1], **4** 0.35 [11], **5** 10 [3], and **7** 0.75 [14]. None of these were detected in uninoculated tissue by column chromatography followed by UV and TLC (detection limit < 100 ng/g fr. wt).

7,2',4'-Trihydroxy-8-(3,3-dimethylallyl)isoflavanone **1** (*5-deoxykievitone*). UV λ_{max}^{MeOH} nm: 286; NaOH 340; $AlCl_3$ 287; $AlCl_3$/HCl 286; NaOAc 262, 286, 340; NaOAc/H_3BO_3 287. ^1H NMR ($CDCl_3$): δ 7.74 (1H, *d*, J = 8.6 Hz, C-5); 7.38 (1H, *d*, J = 8.2 Hz, C-6'); 6.51 (1H, *d*, J = 8.6 Hz, C-6); 6.47 (1H, *d*, J = 2.4 Hz, C-3'); 6.39 (1H, *dd*, J = 8.2, 2.4 Hz, C-5'); 5.25 (1H, *br t*, $J \sim$ 7Hz, C-2''); 5.01 (1H, *dd*, $J \sim$ 12.0, 3.3 Hz, C-2a); 4.81 (1H, *dd*, $J \sim$ 12.0, 4.4 Hz, C-2b); 3.90 (1H, *t*, $J \sim$ 3.6 Hz, C-3); 3.44 (2H, *br d*, $J \sim$ 7.2 Hz, C-1''); 1.85 (3H, *s*, Me); 1.78 (3H, *s*, Me); ($(CD_3)_2CO$): δ 7.63 (1H, *d*, J = 8.6 Hz, C-5); 6.95 (1H, *d*, J = 8.4 Hz, C-6'); 6.64 (1H, *d*, J = 8.6 Hz, C-6); 6.44 (1H, *d*, J = 2.5 Hz, C-3'); 6.31 (1H, *dd*, J = 8.4, 2.5 Hz, C-5'); 5.25 (1H, *t* with long range coupling to the methyls, J = 7.2, 1.4 Hz, C-2''); 4.68 (1H, *dd*, J = 11.0, 9.7 Hz, C-2a); 4.58 (1H, *dd*, J = 11.0 Hz, 5.3 Hz, C-2b); 4.11 (1H, *dd*, J = 9.7, 5.3 Hz, C-3); 3.35 (2H, *d*, J = 7.3 Hz, C-1''), 1.77 (3H, *d*, J = 1.3 Hz, Me), 1.65 (3H, *d*, J = 1.3 Hz, Me). MS: M^+ 340.1314, $C_{20}H_{20}O_5$ requires: 340.1311, *m/e* (rel. int.): 340 (60), 205 (100), 203 (7), 176 (11), 161 (14), 149 (70), 136 (29). The molecular ion showed three exchangeable hydrogens in the presence of D_2O. TLC: R_f 0.40, 0.67, 0.79, 0.28, 0.29, 0.36 in systems I–VI, respectively.

5,7,2',4'-Tetrahydroxy-8-(3,3-dimethylallyl)isoflavone **4** (*2,3-dehydrokievitone*). UV λ_{max}^{MeOH} nm: 265, 286 (sh), 333 (sh); NaOH 281, 330 (sh); $AlCl_3$ 274, 307 (sh), 388; $AlCl_3$/HCl 275, 388; NaOAc 278, 337; NaOAc/H_3BO_3 265. ^1H NMR ($(CD_3)_2CO$): δ 12.69 (1H, *s*, C-5 OH); 8.24 (1H, *s*, C-2); 8.18 (1H, *d*, J = 8.4 Hz, C-6'); 6.49 (1H, *d*, J = 2.4 Hz, C-3'); 6.44 (1H, *dd*, J = 8.4, 2.4 Hz, C-5'); 6.42 (1H, *s*, C-6); 5.26 (1H, *t* with long range coupling to the Me's J = 7.2, 1.3 Hz, C-2''); 3.46 (2H, *br*, *d*, J = 7.2 Hz, C-1''); 1.81 (3H, *d*, J = 1.3 Hz, Me); 1.66 (3H, *d*, J = 1.3 Hz, Me). MS: M^+ 354.1105, $C_{20}H_{18}O_6$ requires: 354.1103, *m/e* (rel. int.): 354 (94), 353 (6), 339 (21), 337 (7), 311 (100), 299 (80), 298 (17), 219 (10), 205 (13), 177 (15), 165 (35), 161 (7), 153 (15), 135 (10), 134 (19). TLC: R_f 0.29, 0.71, 0.72, 0.54, 0.45, 0.53 in systems I–VI, respectively. Treatment of **4** with HCOOH at room temp. for 24 hr followed by chromatography on an LH-20 column eluted with redistilled 95% EtOH gave a single isomer, cyclo-2,3-dehydrokievitone **9**. UV λ_{max}^{EtOH} nm: 266; ^1H NMR ($(CD_3)_2CO$): δ 12.54 (1H, *s*, C-5 OH), 8.28 (1H, *s*, C-2); 7.14 (1H, *d*, J = 8.2 Hz, C-6'); 6.50 (1H, *d*, J = 2.3 Hz, C-3'); 6.45 (1H, *dd*, J = 8.2, 2.4 Hz, C-5'); 6.18 (1H, *s*, C-6); 1.38 (6H, *s*, Me's). (The 4 protons in the heterocyclic 2,2-dimethylchroman ring were obscured by H_2O and Me_2CO.) TLC: R_f 0.43 in system I.

Compound **7** (*1'',2''-dehydrocyclokievitone*). UV λ_{max}^{MeOH} nm: 271, 294 (sh), 307 (sh), 358; NaOH 278, 385; $AlCl_3$ 280, 325, 417; $AlCl_3$/HCl 280, 322, 417; NaOAc 272; NaOAc/H_3BO_3 272. ^1H NMR ($(CD_3)_2CO$): δ 12.49 (1H, *s*, C-5 OH); 6.94 (1H, *d*, J = 8.4 Hz, C-6'); 6.54 (1H, *d*, J = 10.0 Hz, C-1''); 6.46 (1H, *d*, J = 2.4 Hz, C-3'); 6.34 (1H, *dd*, J = 8.4, 2.4 Hz, C-5'); 5.89 (1H, *s*, C-6); 5.63 (1H, *d*, J = 10.0 Hz, C-2''); 4.69 (1H, *t*, J = 10.9 Hz, C-2a); 4.55 (1H, *dd*, J = 10.9, 5.7 Hz, C-2b); 4.29 (1H, *dd*, J = 10.9, 5.8 Hz, C-3); 1.44 (3H, *s*, Me); 1.43 (3H, *s*, Me); MS: M^+ 354.1103 $C_{20}H_{18}O_6$ requires: 354.1103, *m/e* (rel. int.) 354 (73), 353 (11), 339 (100), 337 (20), 321 (30), 219 (25), 218 (23), 217 (45), 203 (90), 169.5 (8), 137 (11), 136 (18). TLC: R_f 0.44, 0.59, 0.55, 0.58 in systems I, IV–VI, respectively.

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