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Unexpected B-ring regioselective di-nitration of diosmetin, a *Citrus* flavonoid

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ABSTRACT

Nitration in position C-8 of diosmetin, an easily available citroflavonoid, was studied in order to gain access to original analogs. The one-step nitration proved impossible, as mono or di-nitration on C-2′ and C-6′ positions on the lateral B ring of the molecule was exclusively observed. This surprisingly straightforward di-nitration of ring B, showing a lack of reactivity of ring A despite its high activation, has never been mentioned to date. Nitration in position C-8 was therefore performed in five steps, requiring selective deactivation of ring B.

Numerous publications describe the preparation of nitroflavones. Some of these compounds have been synthesized for their pharmacological interest, with anxiolytic,[1] cytotoxic,[2] and anti-allergic[3] properties. Others, without remarkable properties, are intermediates toward aminoflavones which have an interest in the fields of cancer,[4] diabetes,[5] and neuroprotection.[6] Different strategies have been described, in which nitro groups are introduced either at the early stages of synthesis, or during the late steps, on the fully constituted flavone skeleton itself. We focused on the latter approach, using diosmetin **1**, a natural citroflavonoid readily accessible from hesperidin or diosmin as a starting material. Our aim was to study conditions of nitration so as to obtain original analogs bearing a substituent in position 8 on the A ring. A thorough examination of the literature concerning the nitration of natural 5,7-dioxygenated flavones showed that the site of nitration (A or B ring) is strongly dependent on the nature of substituents. When the B ring is unsubstituted (5,7-dihydroxyflavone = chrysin **2**), nitration occurs only, as expected, on the A ring, primarily in position 8 then, under stronger conditions, in position 6.[3,5,7] For a monosubstituted B ring, with a hydroxyl group in position 4′ (apigenin **3** and its 7-*O*-neohesperidoside = rhoifolin), mono-nitration is mainly observed at position 3′ of the B ring.[8] However, surprisingly, for a methoxy substitution in 4′ (acacetin **4**), nitration exclusively occurs on the A ring, even after deactivation (acacetin-7-*O*-benzyl-sulfonate leading to a mixture of 6 and 8 mononitrated derivatives).[8]

Nitration of diosmetin with nitric acid (1 or 2 equiv) was conducted in acetic acid (CH_3COOH) or in trifluoracetic acid (TFA). In TFA (1 equiv, HNO_3, 0 °C, 30 min), the reaction medium was homogeneous and yielded, after treatment and flash chromatography, a major product (42%). This compound displayed a molecular peak at *m*/*z* 345 in EIMS, accordingly to a mono-nitration process. However, ^{1}H NMR revealed split signals in a 53:47 ratio, in favor of a mixture of two isomers. In particular, the occurrence of two doublets (J = 8.2 Hz) at 7.30 and 7.48 ppm, assigned to H-5′ and H-6′ and of two singlets at 7.14 and 7.79 ppm corresponding to H-2′ and H-5′ proved them to be 2′-nitrodiosmetin **5a** (major) and 6′-nitrodiosmetin **5b** (minor).[9] In CH_3COOH (HNO_3 1 equiv, 60 °C, 1 h), the reaction medium never cleared. TLC on silica gel showed, in addition to residual diosmetin and mononitrated products, the presence of a bright yellow, highly polar compound. The latter became the largely major product of the reaction performed with 2 equiv of HNO_3 in CH_3COOH (60 °C, 1 h) or in TFA (0 °C, 30 min), while diosmetin and mononitrated compounds were no longer observable. The CH_3COOH reaction, cleaner than that in TFA, allowed isolation of this major compound (58%), identified as 2′,6′-dinitrodiosmetin **6**. The dinitro-substitution was evidenced by EIMS with a molecular peak at *m*/*z* 390. Di-substitution of the B ring was deduced from ^{1}H NMR spectra: H-6 and H-8 signals were still observed at 6.26 and 6.33 ppm (J = 2 Hz). An Overhauser effect between signals of H-5′ and methoxyl group allowed positioning of nitro groups at positions 2′ and 6′. Lastly, 2D NMR provided full structural confirmation for **6**.[10]

MMono-nitration at C-2′ or C-6′, the two activated positions of the B ring, is not surprising in itself. However, mono-nitration would be expected at the A ring for iodination, the only electrophilic substitution described so far on diosmetin, occurred exclusively in positions 6 and 8.[11] In this case, the iodination reagent ($BTMA.ICl_2$) was used in a CH_2Cl_2/MeOH/$CaCO_3$ system. We therefore decided to study the behavior of diosmetin toward

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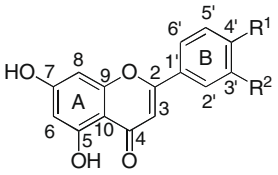
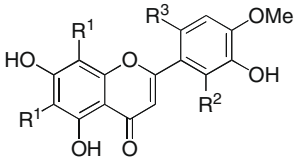
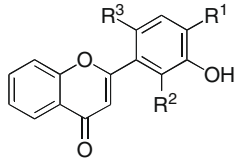


R^1	R^2	
OMe	OH	**1**
H	H	**2**
OH	H	**3**
OMe	H	**4**



R^1	R^2	R^3	
H	NO_2	H	**5a**
H	H	NO_2	**5b**
H	NO_2	NO_2	**6**
Br	H	H	**7**



R^1	R^2	R^3	
H	H	H	**8**
H	NO_2	H	**9a**
NO_2	H	H	**9b**
H	H	NO_2	**9c**

another electrophilic reagent, *N*-bromosuccinimide, under similar conditions as those described for nitration (NBS 1 or 2 equiv, TFA, rt, 1 h). One equivalent of NBS provided a mixture of four compounds: the remaining diosmetin, 6- and 8-bromodiosmetin, and 6,8-dibromodiosmetin **7**,[12] the latter being quantitatively obtained with 2 equiv NBS. Di-nitration at C-2′ and C-6′ appears much more surprising for several reasons: (a) formation of **6** was possible under mild conditions while, in comparison, preparation of 6,8-dinitrochrysin requires 10 h at 65 °C;[5] (b) two nitro groups were introduced on the B ring, despite the presence of a strongly activating 5,7-diphenolic system on the A ring, although a first nitro substitution is well known to deactivate an aromatic ring in regard to a second nitration (flavone itself leads, with excess HNO_3, to a mixture of two dinitroflavones substituted at C-6 on the A ring, and at C-3′ or C-4′ on ring B).[1] We thus wondered whether the exclusive nitration at C-2′ and/or C-6′ of the B ring could result (when 2′ and 6′ are activated as in **1**) from a privileged reactivity of these positions on the B ring. However, that hypothesis was not supported by the nitration reaction of 3′-hydroxyflavone **8** (1 equiv HNO_3, TFA, 0 °C, 30 min) which led to the isolation and the identification of **9a** (23%), **9b** (23%), and **9c** (18%), the three mononitro isomers at 2′, 4′, and 6′, respectively.[13] Looking for the proof of a possible interaction of the first nitro substituent with ring A, we compared A ring proton chemical shifts in mononitro analogs **5a**, **5b,** and **9a**–**c** and in their respective precursors, **1** and **8**. Significant δ variations (shielding) were observed for the sole H-8 signal and only with 2′ or 6′ nitroflavones [−0.23 ppm for **5a** and **5b** vs **1**; −0.30 and −0.19 ppm for **9a** and **9c** vs **8** (but +0.01 ppm for **9b**)], which can be indicative of a diamagnetic anisotropy effect of the 2′ or 6′ nitro group on H-8. In order to verify this possible anisotropic effect of the 2′ or 6′ nitro group on H-8, we performed a GAUSSIAN 03[14] optimization calculation HF/6-31G(d,p) over **1**, **5a,** and **5b** compounds. According to Gaussian results and considering charge distribution we did not find a plausible explanation for these privileged nitrations in positions 2′ or 6′ belonging to ring B. In fact, Gaussian results for compound **1** clearly demonstrate that position 8 should be the first nitration site. However, the distance and position of the nitro groups in respect to H-8 in the optimized structures of **5a** and **5b** shows that nitration at position 2′ or 6′ could have an anisotropic effect on H-8. A similar result showing this anisotropic effect of a nitro group was published by Martin and Nance.[15] So we think that this observed then confirmed through-space effect of the first 2′ or 6′ nitro group on H-8 could be related to the absence of the second nitration at this position.

This attack of diosmetin, at C-2′ and C-6′ only, indicates that nitration on the A ring at C-8 requires a previous deactivation of the B ring. Therefore, our initial objective was reached from diosmetin by the following five-step sequence (Scheme 1): (a) 7-*O* benzylation to **10**;[16] (b) 3′-*O* tosylation to **11** (mp 208–211 °C); (c) debenzylation to **12**;[17] (d) nitration at C-8 to **13**;[18] (e) hydrolysis to 8-nitrodiosmetin **14**.[19] The nitration at C-8 was unambiguously proved by comparison of ^1H and ^{13}C NMR spectra of **1** and **14**: in **14**, deshielding of H-6 (+0.18 ppm) and shielding of C-7 (−6.4 ppm) and C-9 (−7.5 ppm) signals (carbons *ortho* to the nitro group) versus diosmetin.[20]

As a conclusion, this nitro-functionalization at C-8, originally supposed to be a straightforward reaction, appears to be more complex, and highlights an unusual and unknown reactivity within this already well known and well-explored series of flavonoids.

Acknowledgments

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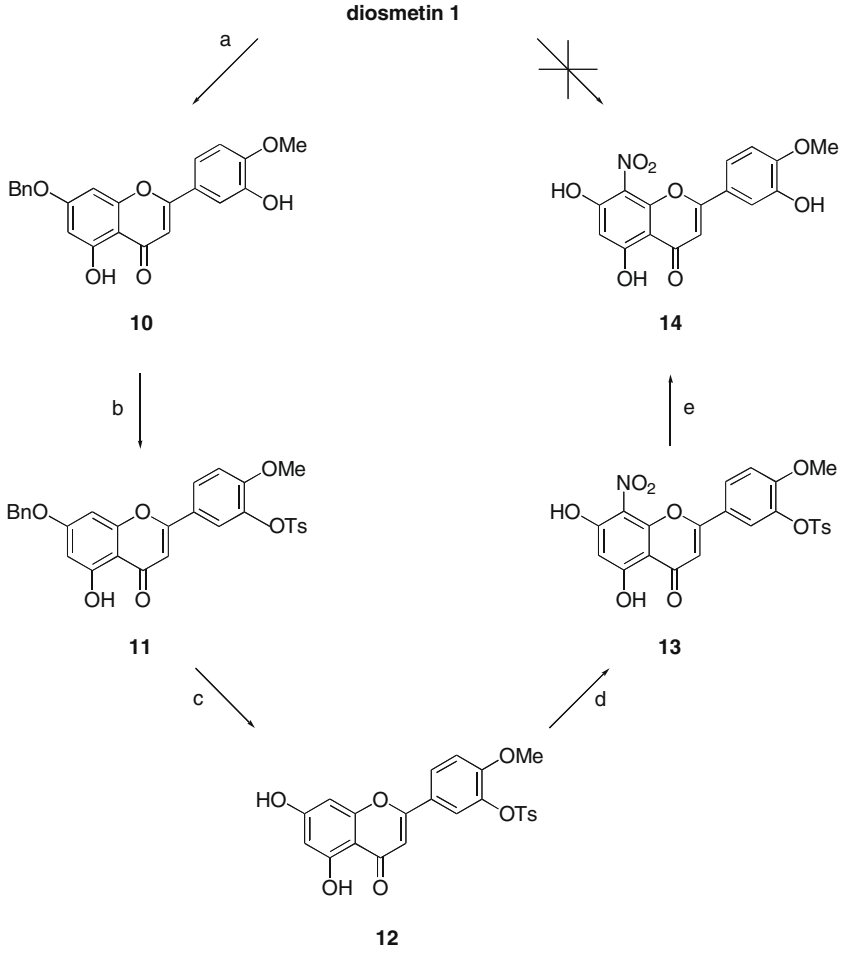
Supplementary data

Supplementary data (^1H NMR spectrum of **5a**/**5b**, ^1H and ^{13}C NMR spectra of **6**, **12**, **13** and **14**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.062.



Scheme 1. Reagents and conditions: (a) $KHCO_3$ 1.1 equiv, benzyl chloride 1.2 equiv, DMF, 120 °C, 2 h, 66%; (b) tosyl chloride 5 equiv, pyridine, rt, 1 h, 93%; (c) H_2, Pd–C 10%, DMF, rt, 2 h, 94%; (d) HNO_3 1 equiv, CH_3COOH, 120 °C, 0.25 h then 60 °C, 1.5 h, 45%; (e) 0.5 N aqueous KOH/MeOH 2/1, 120 °C, 0.5 h, 75%.

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9. Mixture of **5a** (53%) and **5b** (47%). Yellow microcrystalline powder. *Compound* **5a**: ^{1}H NMR ((DMSO-d_6)) δ ppm 4.00 (s, 3H, OMe-4′), 6.19 (d, J = 2.0 Hz, 1H, H-6), 6.23 (d, J = 2.0 Hz, 1H, H-8), 6.72 (s, H-3), 7.30 (d, J = 8.2 Hz, 1H, H-5′), 7.48 (d, J = 8.2 Hz, 1H, H-6′), 12.64 (s, 1H, OH-5); *compound* **5b**: 3.98 (s, 3H, OMe), 6.19 (d, J = 2.0 Hz, 1H, H-6), 6.23 (d, J = 2.0 Hz, 1H, H-8), 6.54 (s, H-3), 7.14 (s, H-2′), 7.79 (s, H-5′), 12.70 (s, 1H, OH-5). HREIMS (+) m/z [M]$^+$ 345.0489 (calcd for $C_{16}H_{11}NO_8$, 345.0485).
10. *Compound* **6**. Beige yellowish powder: mp: 275–277 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ ppm 4.05 (s, 3H, OMe-4′), 6.26 (d, J = 2.0 Hz, 1H, H-6), 6.33 (d, J = 2.0 Hz, 1H, H-8), 6.45 (s, 1H, H-3), 8.00 (s, 1H, H-5′), 12.4 (br s, 1H, OH-5). ^{13}C NMR (DMSO-d_6) δ ppm 57.4 (OMe-4′), 94.0 (C-8), 99.5 (C-6), 103.7 (C-10), 109.7 (C-5′), 110.3 (C-3), 114.0 (C-1′), 136.7 (C-6′), 139.1 (C-2′), 146.2 (C-3′), 150.8 (C-4′), 157.8 (C-2 and C-9), 161.5 (C-5), 165.0 (C-7), 180.9 (C-4). HREIMS (+) m/z [M]$^+$ 390.0342 (calcd for $C_{16}H_{10}N_2O_{10}$, 390.0336).
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12. Quintin, J.; Lewin, G. *Tetrahedron Lett.* **2005**, *46*, 4341–4343.
13. The position of the nitro group in each isomer was proved by comparative ^{1}H NMR experiments (NOESY and COSY). The conclusions were based on: (a) the presence of Overhauser effects between H-3 and H-2′/H-6′ (two NOE observed for **9b**, only one for **9a** and **9c**); (b) the strong deshielding of H-5′ signal by the *ortho* nitro group at 4′ for **9b** and 6′ for **9c**. *Compound* **9a**. White crystals: mp: 231–233 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ ppm 6.78 (s, 1H, H-3), 7.29 (d, J = 8.4 Hz, 1H, H-4′), 7.36 (d, J = 7.7 Hz, 1H, H-6′), 7.45 (d, J = 8.4 Hz, 1H, H-8), 7.50–7.58 (m, 2H, H-6 and H-5′), 7.83 (br t, 1H, H-7), 8.05 (d, J = 7.9 Hz, 1H, H-5). *Compound* **9b**. White crystals: mp: 238–240 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ ppm 7.08 (s, 1H, H-3), 7.53 (br t, J = 7.5 Hz, 1H, H-6), 7.68 (br d, J = 8.7 Hz, 1H, H-6′), 7.75–7.79 (m, 2H, H-8 and H-2′), 7.86 (br t, 1H, H-7), 8.03 (d, J = 8.7 Hz, 1H, H-5′), 8.06 (d, J = 7.9 Hz, 1H, H-5). *Compound* **9c**. White crystals: mp: 279–282 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ ppm 6.63 (s, 1H, H-3), 7.08–7.13 (m, 2H, H-4′ and H-2′), 7.52 (t, J = 7.7 Hz, 1H, H-6), 7.56 (d, J = 8.5 Hz, 1H, H-8), 7.81 (br t, 1H, H-7), 8.08 (d, J = 7.8 Hz, 1H, H-5), 8.16 (d, J = 8.4 Hz, 1H, H-5′).
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17. *Compound* **12**. Yellowish powder: mp: 244–246 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ ppm flavone moiety: 3.62 (s, 3H, OMe-4′), 6.20 (d, J = 2.0 Hz, 1H, H-6), 6.37 (d, J = 2.0 Hz, 1H, H-8), 6.80 (s, 1H, H-3), 7.21 (d, J = 8.9 Hz, 1H, H-5′), 7.54 (d, J = 2.0 Hz, 1H, H-2′), 8.00 (dd, J = 8.9 and 2.0 Hz, 1H, H-6′), 10.9 (br s, 1H, OH-7), 12.80 (s, 1H, OH-5); tosyl moiety: 2.45 (s, 3H, Me-4″), 7.49 (d, J = 8.9 Hz, 2H, H-3″ and 5″), 7.75 (d, J = 8.9 Hz, 2H, H-2″ and 6″). ^{13}C NMR (DMSO-d_6) δ ppm flavone moiety: 56.1 (OMe-4′), 93.9 (C-8), 98.9 (C-6), 103.7 (C-10), 104.3 (C-3), 113.8 (C-5′), 121.4 (C-2′), 122.9 (C-1′), 127.0 (C-6′), 145.8 (C-3′), 154.3 (C-4′), 157.1 (C-9), 161.4 (C-2), 161.5 (C-5), 164.3 (C-7), 181.6 (C-4); tosyl moiety: 21.1 (Me), 128.4 (C-2″ and 6″), 129.9 (C-3″ and 5″), 131.8 (C-1″), 137.7 (C-4″). EIMS (+) m/z [M]$^+$ 454.

18. *Compound* **13**. Yellowish powder: mp: 266–267 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ ppm flavone moiety: 3.60 (s, 3H, OMe-4′), 6.37 (s, 1H, H-6), 7.07 (s, 1H, H-3), 7.27 (d, J = 8.9 Hz, 1H, H-5′), 7.68 (d, J = 2.0 Hz, 1H, H-2′), 7.95 (dd, J = 8.9 and 2.0 Hz, 1H, H-6′), 13.32 (s, 1H, OH-5); tosyl moiety: 2.45 (s, 3H, Me-4″), 7.48 (d, J = 8.9 Hz, 2H, H-3″ and 5″), 7.74 (d, J = 8.9 Hz, 2H, H-2″ and 6″). ^{13}C NMR (DMSO-d_6) δ ppm flavone moiety: 56.1 (OMe-4′), 98.9 (C-6), 102.9 (C-10), 105.1 (C-3), 114.0 (C-5′), 121.3 (C-8), 121.7 (C-2′), 122.2 (C-1′), 127.2 (C-6′), 145.8 (C-3′), 149.8 (C-9), 154.7 (C-4′), 157.9 (C-7), 161.8 (C-2), 162.7 (C-5), 181.1 (C-4); tosyl moiety: 21.1 (Me), 128.2 (C-2″ and 6″), 129.9 (C-3″ and 5″), 131.8 (C-1″), 137.8 (C-4″). EIMS (+) m/z $[M]^+$ 499.

19. *Compound* **14**. Yellow crystals: mp: 303–306 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ ppm 3.88 (s, 3H, OMe-4′), 6.37 (s, 1H, H-6), 6.96 (s, 1H, H-3), 7.10 (d, J = 8.9 Hz, 1H, H-5′), 7.37 (d, J = 2.0 Hz, 1H, H-2′), 7.48 (dd, J = 8.9 and 2.0 Hz, 1H, H-6′), 13.40 (s, 1H, OH-5). ^{13}C NMR (DMSO-d_6) δ ppm 55.9 (OMe-4′), 98.8 (C-6), 102.9 (C-10), 104.2 (C-3), 112.3 (C-5′), 113.1 (C-2′), 119.0 (C-6′), 121.5 (C-8), 122.2 (C-1′), 146.9 (C-3′), 149.8 (C-9), 151.7 (C-4′), 157.8 (C-7), 162.7 (C-5), 163.7 (C-2), 181.1 (C-4). EIMS (+) m/z $[M]^+$ 345.

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