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Studies on the synthesis of safflomin-A, a yellow pigment in safflower petals: oxidation of 3-*C*-β-D-glucopyranosyl-5-methylphloroacetophenone

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Abstract—The direct C-glycosylation of methylphloroacetophenone **8** with D-glucose gave *C*-β-D-glucopyranosylmethylphloroacetophenone (**7**) in 65% yield, which, on oxidation in the presence of small amount of pyridine under an oxygen atmosphere afforded the quinone **9**, oxidized at the methylated position of the benzene ring as a pair of diastereomers in 27% yield. A detailed NMR analysis and a comparison of the UV–vis and CD spectra of their acetates indicated that the structure and stereochemistry of **9** was (1*R*,1′*S*,2*R*,3*S*,3a*S*,5*R* and 1*R*,1′*S*,2*R*,3*S*,3a*S*,5*S*)-7-acetyl-2-(1′,2′-dihydroxyethyl)-5-methyl-3,5,6-trihydroxy-8-oxofuro[3,2-*d*]-benzo[*b*]furan.

Keywords: Safflower; Yellow pigment; Safflomin-A; Direct C-glycosylation; Oxidation

1. Introduction

The following yellow and red pigments produced in Safflower (*Carthamus tinctrious* L.) petals have been reported: (1) a red pigment: carthamin;[1] (2) a yellow pigment: a carthamin precursor;[2] safflower yellow B;[3] safflomin-A[4a–c] and –C;[5] tinctromine[6] and cartormin.[7] All these pigments contain a characteristic *C*-β-D-glucopyranosylquinochalcone structure. The structures of these pigments have been elucidated. However, in the case of one of the yellow pigments, safflomin-A, three different structures (**1**, **2**, and **3** in Fig. 1) have been proposed. The major difference between these structures is the nature of the glucosyl moiety linked to the α position, between the diketone carbons. One of them, a *C*-β-D-glucopyranosyl structure **3** recently proposed by Goda et al.[4c] appears to be the valid structure. The other two proposed structures (**1** and **2**) are quite different. It is assumed that these different assignments for safflomin-A are partially due to the instability of the compound due to *C*-β-D-glucopyranosyl moiety linked to the α carbon between the 1,3-diketone of the characteristic quinochalcone.

2. Results and discussion

The model compound **4** in which the two glucosyl groups are replaced by methyl groups has already been synthesized (see Fig. 2).[8] Our interest was focused on the synthesis of the more natural model compound **5**, in an attempt to verify the proposed structure of safflomin-A. The model compound **5** contains a *C*-β-D-glucopyranosyl moiety linked to the α carbon between the 1,3-diketone carbons of quinochalcone. The retrosynthetic analysis of **5** is shown in Scheme 1. We wish to report here on an attempt to synthesize the key compound **6** for the synthesis of **5**. Methylphloroacetophenone **8** was synthesized via the acetylation of phloroglucinol by the Hoesch reaction, followed by formylation by the Gattermann reaction and the subsequent conversion

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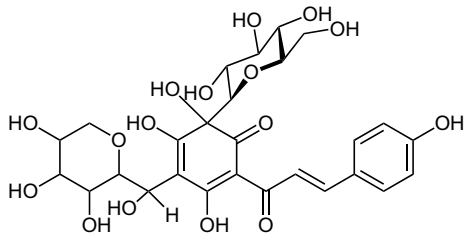

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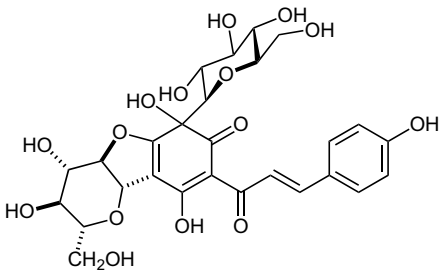

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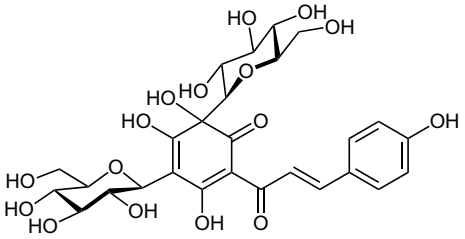

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Figure 1. Proposed structures for safflomin-A.

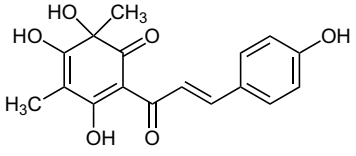

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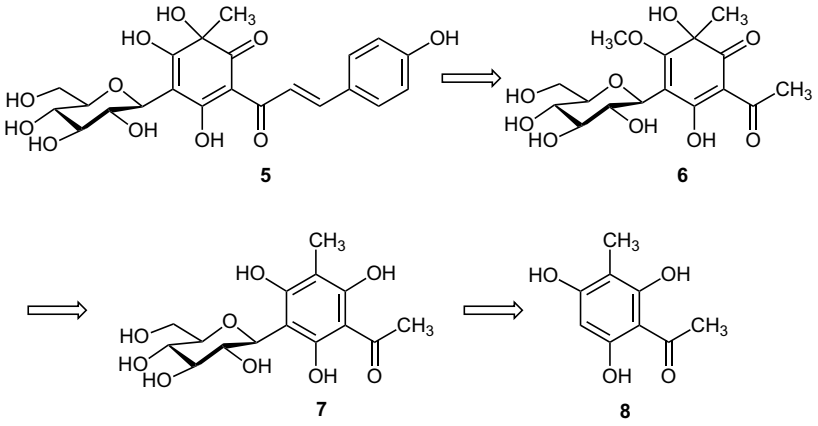
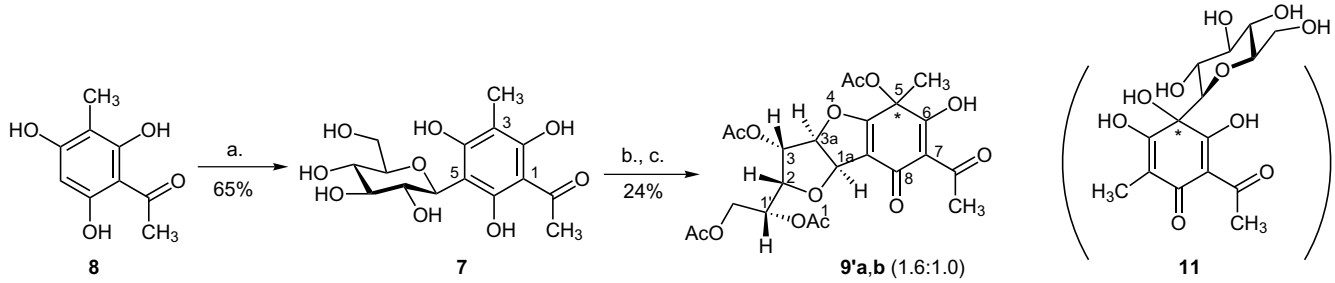
Figure 2. Model compound for safflomin-A.

of the formyl group into a methyl group by a Clemennsen reduction in a total yield of 42.5%.[9] Since the C-glycosylation of **8** using Suzuki's O→C glycoside rearrangement method was not successful,[10] we employed a direct C-glycosylation using unprotected D-glucose in the presence of a catalytic amount of scandium(III) trifluoromethanesulfonate ($Sc(OTf)_3$) in aqueous media, a procedure that was recently developed in our laboratory.[11] The direct C-glycosylation of **8** was achieved by dissolving 3 equiv of D-glucose in 1:1 acetonitrile–water in the presence of 0.2 equiv of $Sc(OTf)_3$, followed by refluxing for 13 h to afford the *C*-β-D-glucoside **7** in 65% yield, with a recovery of 25%. Extension of the refluxing time or the addition of $Sc(OTf)_3$ gave more reaction byproducts such as spiro-compounds via the dehydration of **7**.[12a–c]

The oxidation of **7** was next examined. Since we successfully used an air-oxidation method using $Pb(OAc)_2$ as a catalyst in the synthesis of the safflomin-C model compound,[13] the same reaction was used here. However, *C*-glycoside **7** failed to react under these conditions. Other oxidation conditions in which metals such as Mn(IV) and W(VI) are used as catalysts also led to no reaction. However, simple air- or oxygen-oxidation without any added oxidizing reagents in methanol in the presence of a small amount of pyridine proceeded very slowly. Vigorous stirring of a methanolic solution of **8** in the presence of 2 equiv of pyridine under an oxygen atmosphere at room temperature for 3 days afforded the oxidized products **9a** and **9b** in a total yield of 27%. When the amount of added pyridine was increased to 5 or 10 equiv, the reaction time was shorter, but the yield remained the same. Because **9a** and **9b** were not separable, they were acetylated in the usual manner. The acetates **9′a** and **9′b** could be separated by silica-gel column chromatography (**9′a**:**9′b** = 1.6:1.0). Since **9′a** and **9′b** showed analogous NMR spectra, and a characteristic single peak derived from the triketo structure was observed at the downfield position of 18.4 ppm, the presence of the oxidized quinone skeleton could be confirmed. Furthermore, it was clear from the ^1H NMR spectra that the glucose moiety was not in the expected chair form like **7**. By further ^1H–^1H COSY, ^1H–^{13}C COSY, HMBC, and NOESY experiments, the structures of **9′a** and **9′b** were assigned as 3,5-acetoxy-7-acetyl-2-(1′,2′-diacetoxyethyl)-6-hydroxy-5-methyl-8-oxofuro[3,2-*d*]benzo[*b*]furan, as shown in Scheme 2, in which the dehydrated ring-closure of a phenolic hydroxy group at the *ortho*-position and the 2-hydroxy group of the glucose moiety, and further ring-closure between the anomeric carbon and the 4-hydroxyl in the glucose moiety occurred. The possibility of the formation of pyro[3,2-*d*]benzo[*b*]furan was deemed negligible, based on the upfield shift of the H-2 (H-2: **9′a**, 3.77 ppm; **9′b**, 3.89 ppm; H-1′: **9′a**, 5.31 ppm; **9′b**, 5.28 ppm). It is also possible that each acetate **9′a** and **9′b** is a regioisomer formed by dehydration of a 2-positioned hydroxyl of the glucose and the phenolic hydroxyl group at the 6-position. However, the UV–vis spectra of **9′a** and **9′b** matched, and their CD spectra were symmetric (Fig. 3). Furthermore, in a comparison of the CD spectra of (4*S* and 4*R*)-2-acetyl-3,4-dihydroxy-5-methoxy-4-methyl-2,5-cyclohexadienones (**10a** and **10b**),[14] each CD spectrum of **9′a** and **9′b** was consistent with that of **10b** and **10a**, respectively. The above experiments indicate that they were diastereomers derived from the difference in the stereochemistry at the 5-carbinol carbon, and the stereochemistry of the 5-positioned carbinol-carbon atom of **9′a** and **9′b** was determined to be *R* and *S*,



Scheme 1. Retrosynthetic analysis of model compound **5**.



Scheme 2. Reagents and conditions: (a) D-glucose (3 equiv), $Sc(OTf)_3$ (0.2 equiv), in 1:1 CH_3CN–H_2O, reflux, 13 h; (b) O_2–Py (2 equiv) in MeOH, rt for 3 days; (c) Ac_2O–Py.

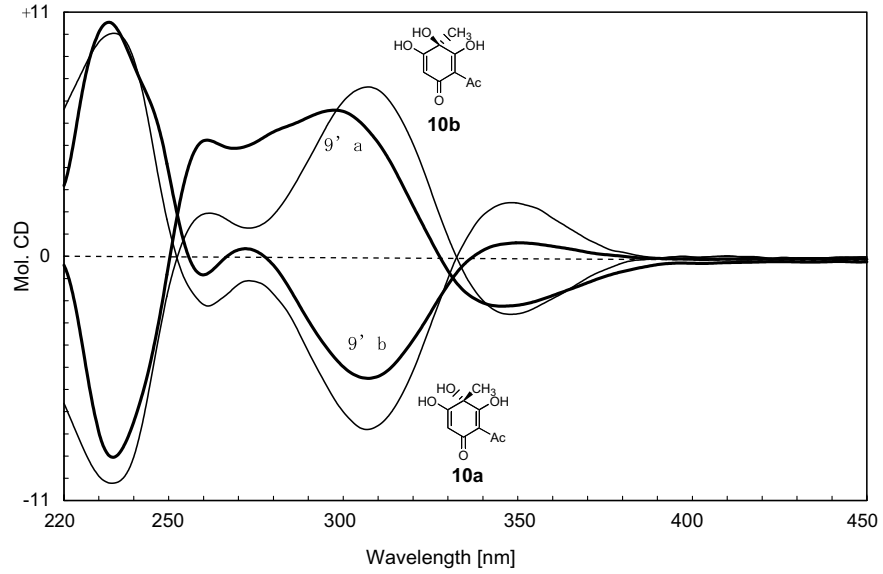
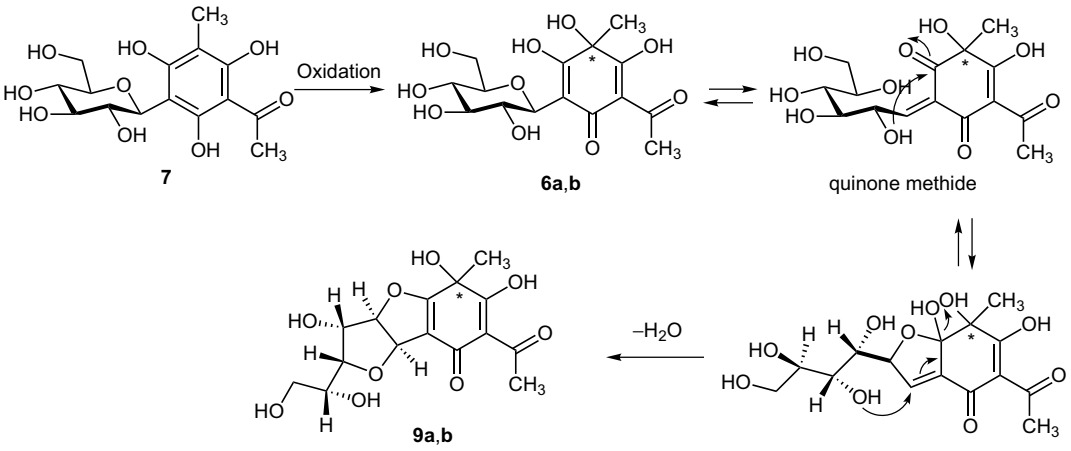


Figure 3. CD spectra of **9′a**,**b** and **10a**,**b**.

respectively. It was assumed from the elucidated structure of **9′a** and **9′b** that the formation of **9** might occur by the proposed mechanism shown in Scheme 3. *C*-Glycoside **7** is oxidized to form the desired quinone diastereomers **6a** and **6b**, which are unstable and underwent spontaneous dehydration via the quinone methide.[15] That is, the 2-hydroxy group's oxygen of the glucose moiety nucleophilically attacked the *ortho*-positioned



Scheme 3. Proposed mechanism for the formation of **9** by the oxidation of **7**, followed by dehydration and ring closure.

carbonyl-carbon formed by oxidation, followed by dehydration to form a mixture of oxidized benzo[*b*]furan diastereomers, and subsequently underwent recyclization by the 4-hydroxy group of the glucose moiety to give the furo[3,2-*d*]benzo[*b*]furans **9a** and **9b**. Under these oxidation conditions, the formation of **11** oxidized at the C-glycosylated 5-position in **6** was not observed. Quinone **9** is analogous to one of the three proposed structures for safflomin-A (**2**).[4b] It was found that the *C*-β-D-glucopyranosylquinone produced by oxidation was unstable and the 2-hydroxy group of its glucose moiety readily reacted with an *ortho*-carbonyl carbon under mild conditions. In fact, silica-gel TLC confirmed that natural safflomin-A is unstable, and its structure is altered in the presence of a small amount of trifluoroacetic acid in methanol at room temperature after 1 day. Therefore, it is clear that the glucopyranosyl hydroxy group of **7** should be protected during the formation of the desired quinone **6** by oxidation.

3. Experimental

3.1. General

The solvents used in this reaction were purified by distillation. For separation and purification, flash column chromatography was performed on silica gel (230–400 mesh, Fuji-Silysia Co., Ltd., BW-300). Melting points were determined on a Shibayama micro-melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-370 polarimeter. Mass spectral data were obtained by fast-atom bombardment (FAB) using 3-nitrobenzyl alcohol (NBA) as a matrix on a JEOL JMS-AX505HA instrument. IR spectra were recorded on a Horiba FT-720 IR spectrometer. NMR spectra were recorded on a Varian Inova 500 spectrometer using Me_4Si as the internal standard. Circular dichroism (CD) spectra were recorded on a JASCO J-720WI spectropolarimeter. Elemental analyses were performed on a Perkin–Elmer PE 2400 II instrument.

3.2. 3-*C*-β-D-Glucopyranosyl-5-methylphloroacetophenone (7)

Methylphloroacetophenone **8** (500 mg, 2.747 mmol), D-glucose (1.484 g, 8.242 mmol), and $Sc(OTf)_3$ (270 mg, 0.549 mmol) were dissolved in 1:1 CH_3CN–H_2O (13 mL) and stirred while refluxing in an oil bath at 80 °C under Ar for 13 h. The reaction mixture was evaporated in vacuo, and the residue was separated by flash column chromatography on silica gel (20:1 and 5:1 $CHCl_3$–MeOH) to afford recovered **8** (125 mg, 25%) and the glycoside **7** (614 mg, 65%) as a pale-brown solid. Glycoside **7** was recrystallized from CH_3CN to give colorless prisms: mp 134–135 °C. $[\alpha]_D^{21}$ +106 (*c* 1.135, $CHCl_3$); IR (KBr): *v* 3354, 2931, 1622, 1437, 1367, 1271, 1184, 1136, 1101, and 1026 cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 1.92 (3H, s, CH_3), 2.60 (3H, s, Ac), 3.29 (1H, d, *J* 9.5 Hz, H-4′), 3.30 (1H, dt, *J* 3.0 and 9.5 Hz, H5′), 3.36 (1H, t, *J* 9.5 Hz, H-3′), 3.39 (1H, t, *J* 9.5 Hz, H-2′), 3.63 (2H, d, *J* 3.0 Hz, H-6′a,b), 4.79 (1H, d, *J* 9.5 Hz, H-1′), 9.25, 10.70, and 12.60 (each 1H, br s, OH × 3); ^{13}C NMR (Me_2SO-d_6): δ 203.3 (C=O), 161.0, 160.6, and 158.5 (C-2, -4, -6), 105.1 (C-1), 103.7 (C-3), 102.9 (C-5), 32.8 ($COCH_3$), and 18.4 (CH_3), (glucose moiety) 80.9 (C-5′), 77.6 (C-3′), 75.1 (C-1′), 72.7 (C-2′), 68.8 (C-4′), 59.6 (C-6′); FABMS (NBA): *m*/*z* 345 $(M+H)^+$. Anal. Calcd for $C_{15}H_{20}O_9 \cdot 0.5CH_3CN$: C, 52.67; H, 5.94; N, 1.92. Found: C, 52.61; H, 6.10; N, 1.94.

3.3. (1*R*,1′*S*,2*R*,3*S*,3a*S*,5*R* and 1*R*,1′*S*,2*R*,3*S*,3a*S*,5*S*)-3,5-Acetoxy-7-acetyl-2-(1′,2′-diacetoxyethyl)-6-hydroxy-5-methyl-8-oxofuro[3,2-*d*]benzo[*b*]furan (9′a,b)

Pyridine (123 μL, 1.52 mmol) was added to a solution of glycoside **7** (261 mg, 0.759 mmol) in MeOH (5 mL), and

the solution was stirred vigorously for 3 days at room temperature under an oxygen atmosphere. Toluene (30 mL) was added to the reaction mixture, and the solution was then evaporated in vacuo to remove the organic solvents, including the pyridine. The residue was separated by flash column chromatography on silica gel (20:1 and 5:1 $CHCl_3$–MeOH) to give **9** (70 mg, 27%) as a pale-yellow solid. The oxidized product **9** (70 mg, 0.21 mmol) was dissolved in Ac_2O (1 mL) and pyridine (0.7 mL), and the mixture was stirred under an Ar atmosphere at room temperature for 16 h. To the stirred reaction mixture, 1 M HCl (30 mL) was added, and the solution was extracted twice with EtOAc. The combined organic layer was washed with brine and dried over anhyd Na_2SO_4. The organic solvent was then evaporated in vacuo to give a pale-yellow oil that was separated by flash column chromatography on silica gel (3:1 and 1:1 hexane–EtOAc) to give **9′a** (57.1 mg) and **9′b** (35.7 mg) in a total yield of 89% as a pale-yellow viscous oils.

3.3.1. Physicochemical data for 9′a. TLC: R_f 0.41 (1:1 hexane–EtOAc); HPLC: t_R 4.60 min (7:3 MeOH–25% aq AcOH); $[\alpha]_D^{25}$ +85.9 (*c* 1.02, $CHCl_3$); UV–vis (MeOH, log ε): λ 245 (4.22), 283 (3.98), 307 (4.00) nm; CD (MeOH) λ_{ext} (Δε): 233 (−9.31), 262.5 (+5.29), 296.5 (+6.67), 345.5 (−2.34) nm; IR (KBr): *v* 3629, 3483, 3022, 2995, 2937, 1755, 1680, 1653, 1539, 1473, 1371, 1232, 1060, and 758 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.68 (3H, s, CH_3), 2.02, 2.03, 2.12, and 2.14 (each 3H, s, OAc × 4), 2.57 (3H, s, ArAc), 3.77 (1H, dd, *J* 9.0 and 3.5 Hz, H-2), 4.12 (1H, dd, *J* 6.5 and 12.5 Hz, H-2′a), 4.56 (1H, dd, *J* 2.5 and 12.5 Hz, H-2′b), 5.10 (1H, d, *J* 6.0 Hz, H-3a), 5.31 (1H, ddd, *J* 9.0, 6.5, and 2.5 Hz, H-1′), 5.54 (1H, d, *J* 3.0 Hz, H-3), 5.82 (1H, d, *J* 6.0 Hz, H-1a), 18.5 (1H, s, OH); ^{13}C NMR ($CDCl_3$): δ 24.2 (CH_3), 26.4 ($CO\mathit{CH}_3$), 63.3 (C-2′), 67.5 (C-1′), 73.8 (C-3), 75.7 (C-5), 76.3 (C-2), 80.9 (C-1a), 91.5 (C-3a), 107.2 (C-7), 107.4 (C-8a), 175.8 (C-5a), 185.9 (C-6), 190.3 (C-8), 199.4 ($\mathit{C}{=}\mathit{O}CH_3$). FABMS (NBA): *m/z* 511 $(M+H)^+$, 494 (M−16), 331. Anal. Calcd for $C_{23}H_{26}O_{13}{\cdot}0.5H_2O$: C, 53.78; H, 5.45. Found: C, 53.77; H, 5.74.

3.3.2. Physicochemical data for 9′b. TLC: R_f 0.35 (1:1 hexane–EtOAc); HPLC: t_R 4.37 min (7:3 MeOH–25% aq AcOH); $[\alpha]_D^{26}$ +21.8 (*c* 1.01, $CHCl_3$); UV–vis (MeOH, log ε): λ 245 (4.24), 283 (3.87), 307 (3.91) nm; CD (MeOH) λ_{ext} (Δε): 232 (+10.6), 271 (−0.41), 307.5 (−5.53), 350.5 (+0.65) nm; IR (KBr): *v* 3629, 3483, 3022, 2995, 2937, 1755, 1680, 1653, 1539, 1473, 1371, 1232, 1060, and 758 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.68 (3H, s, CH_3), 2.00, 2.02, 2.12, and 2.18 (each 3H, s, OAc × 4), 2.56 (3H, s, ArAc), 3.89 (1H, dd, *J* 9.0 and 3.5 Hz, H-2), 4.17 (1H, dd, *J* 6.2 and 12.5 Hz, H-2′a), 4.47 (1H, dd, *J* 2.4 and 12.4 Hz, H-2′b), 5.13 (1H, d, *J* 6.2 Hz, H-3a), 5.28 (1H, ddd, *J* 9.0, 6.5, and 2.5 Hz, H-1′), 5.37 (1H, d, *J* 2.8 Hz, H-3), 5.86 (1H, d, *J* 6.0 Hz, H-1a), 18.4 (1H, s, OH); ^{13}C NMR ($CDCl_3$): δ 23.8 (CH_3), 26.2 ($CO\mathit{CH}_3$), 63.4 (C-2′), 67.8 (C-1′), 74.4 (C-3), 75.6 (C-5), 75.6 (C-2), 80.3 (C-1a), 91.7 (C-3a), 107.1 (C-7), 107.3 (C-8a), 175.3 (C-5a), 186.0 (C-6), 190.8 (C-8), 199.1 ($\mathit{C}{=}\mathit{O}CH_3$); FABMS: (NBA): *m/z* 511 $(M+H)^+$, 494 (M−16), 331. Anal. Calcd for $C_{23}H_{26}O_{13}{\cdot}0.5H_2O$: C, 53.78; H, 5.45. Found: C, 53.52; H, 5.72.

Acknowledgements

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