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A Facile Synthesis of Emodin Derivatives, Emodin Carbaldehyde, Citreorosein, and Their 10-Deoxygenated Derivatives and Their Inhibitory Activities on μ-Calpain

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A new procedure for the preparation of emodin carbaldehyde and citreorosein was described, in which, ω,ω'-dibromomethylemodin triacetate was prepared as a key intermediate by NBS-mediated bromination of 1,3,8-triacetylemodin. Reduction of emodin and citreorosein with $SnCl_2$ in a 1:1 mixture of HOAc and HCl afforded the corresponding anthrones in 90% and 92% yield, respectively, while the corresponding 10-desoxyemodin carbaldehyde was prepared by MnO_2 oxidation of 10-desoxycitreorosein. 10-Desoxycitreorosein and emodin carbaldehyde showed feasible μ-calpain inhibitory activities with IC_{50} values of 20.15 and 25.77 M, respectively.

Key words: Emodin, Emodin carbaldehyde, Citreorosein, ω,ω'-Dibromomethylemodin, μ-Calpain, Anthrone

INTRODUCTION

Emodin (1,3,8-trihydroxy-6-methylanthracene-9,10-dione, **1a**) was first isolated from Extractum Rhei, powdered extract of herbal medicine rhubarb (*Rhamnus frangula*), as deep orange-colored prismatic crystals (De La Rue and Müller, 1858). However, confirmation of its molecular formula, $C_{15}H_{10}O_5$, took two more decades and a couple of corrections (Liebermann and Waldstein, 1876). The present structure was finally established in 1912 and confirmed by synthesis after lengthy dispute (Oesterle, 1912). It should be noted that a practical isolation procedure was only established in the early 1980s (Kelly et al., 1983).

Studies on the biological properties of crude and pure emodin revealed that it has antibacterial (Ubbink-Kok et al., 1986; Wang and Chung, 1997), antiviral (Cohen et al., 1996), anti-inflammatory (Chang et al., 1996; Di Napoli, 1998), T- and B-cell immunosuppressive (IC_{50} = 0.2 mg/mL) (Huang et al., 1992), vasorelaxant (Sato et al., 2000), antiulcerogenic (Goel et al., 1991), and anticancer activities (Koyama et al., 1989; Srinivas et al., 2003; Yan et al., 2012). Furthermore, inhibitory activities on matrix metalloproteinase-9 (MMP-9) (Wierzchacz et al., 2009), protein tyrosine kinase (Jaysuriya et al., 1992), COX-2 and 5-LOX (Chang and Son, 2011) of emodin have also been reported.

Such a variety of biological properties have led to the development of a few new synthetic methods (Krohn, 1980; Bloomer et al., 1993; Khan et al., 1994) since the first preparation from 3,5-dinitrophthalic anhydride and *m*-cresol (Eder and Widmer, 1923). In addition, syntheses of emodin derivatives have also been pursued on a couple of occasions (Eder and Hauser, 1925; Cameron and Crossley, 1977).

Among the various derivatives of emodin, ω-hydroxyemodin (**2a**, citreorosein), emodin carbaldehyde (**3a**) and emodin carboxylic acid (emodic acid, **4a**) have been attractive compounds. Not only due to being important intermediates for the preparation of various derivatives of emodin, but also as substrates for the preparation of various hypericines (Waser and Falk, 2006), and several complex natural products such as

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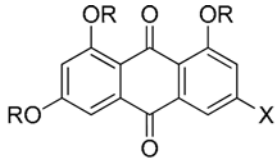
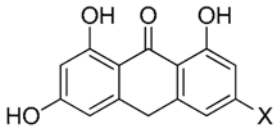


1a R = H, X = CH_3; **1b** R = CH_3, X = CH_3; **1c** R = Ac, X = CH_3
2a R = H, X = CH_2OH
3a R = H, X = CHO; **3b** R = CH_3, X = CHO
4a R = H, X = COOH



5a X = CH_3

Fig. 1. Structures of emodin and its derivatives

nalgiovensin (Banville and Prassard, 1976), endocrocin (Waser et al., 2005), and related natural products.

Citreorosein (**2a**) was first isolated as ω-hydroxyemodin from *Penicillium cyclopium* (Anslow et al., 1940) and as citreorosein from *Penicillium cytreo-rosein* (Posternak and Jacob, 1940). It was later synthesized (Rajagopalan and Seshadri, 1956) in four steps from emodin in 29%, which could be improved up to 48% (Hirose et al., 1982).

Although several synthesis methods for emodin aldehyde have been reported, most of them are inconvenient, multi-step, and/or low-yielding. The original three-step methods involved a conversion of emodic acid (**4a**) to the corresponding acid chloride, followed by reduction in the presence of $Pd/BaSO_4$ to yield the corresponding aldehyde in only up to 10% yield (Murakami, 1956). Modification of this method, by employing BH_3-reduction of emodic acid triacetate to the corresponding alcohol followed by Swern oxidation with DMSO/oxalyl chloride somewhat improved yield (Kim et al., 1997). Two-step synthesis involving CrO_3-oxidation of emodin in Ac_2O/HOAc to the corresponding 6,6-diacetate and subsequent hydrolysis yielded the aldehyde in 11% yield. While five-step synthesis, acetylation by Ac_2O to tri-*O*-acetoxyemodin, NBS-mediated bromination of the 6-methyl group to the 3-bromomethyl analogue, solvolysis by Ac_2O/NaOAc to the 3-acetoxy derivative, acid-catalyzed hydrolysis, and followed by MnO_2 oxidation afforded the aldehyde in 27% overall yield (Thiem and Wessel, 1985). Recently, Salama et al. introduced two additional methods for the preparation of 1,3,8-trimethoxyemodin carbaldehyde (**3b**), in which NBS-mediated dibromination of 1,3,8-trimethoxyemodin (**1b**) and subsequent Ag(I)-mediated hydrolysis of 6,6-dibromomethyl-1,3,8-trimethoxyemodin in resulted in a 67% yield, and hydrolysis of Sommelet salts from monobromo compound produced the corresponding aldehyde (**3b**) in 36% yield (Salama et al., 2003) from emodin.

Emodin anthrone (**5a**), a deoxygenated derivative of **1a**, was initially prepared chemically by refluxing 8-hydroxy-6-methyl-5-bromo-1,3-dimethoxyanthraquinone with a mixture of HOAc and HI (Jacobson and Adams, 1924) and later isolated from the fruit of *Rhamnus dahurica* (Tsukida, 1954), *Rheum palmatum* (Lemli et al., 1964), *Cassia rogeoni* (Haag-Berrurier et al., 1977), *C. nomame* (Kitanaka and Takido, 1985), and *Dermocybe* sp. (Gill and Morgan, 2001).

Our recent study on screening biologically active compounds from natural sources revealed that emodin-related compounds might be a good lead for anti-Alzheimer agents. We herein described a general and efficient synthesis method for emodin carbaldehyde, citreorosein and related compounds, as well as the results of their inhibitory activities against μ-calpain.

MATERIALS AND METHODS

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. IR spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz or 400 MHz for ^{1}H-NMR and 62.5 MHz or 100 MHz for ^{13}C-NMR and are reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). Chemicals and solvents were commercial grade reagents, used without further purification. Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer. Although emodin can be prepared by employing previously reported methods (Jacobson and Adams, 1924; Krohn, 1980), emodin is now commercially available from several sources. μ-Calpain (human erythrocyte) was purchased from Calbiochem. MDL28170 and E64d were purchased from Sigma. Pep1, a substrate of μ-calpain, was synthesized by the Peptron Corp. Pep1 was derived from the p35 cleavage site ([2-Abz]-Ser-Thr-Phe-Ala-Gln-Pro-[3-nitrotyrosine]-NH_2).

1,3,8-Triacetoxy-6-methylanthracene-9,10-dione (1,3,8-Triacetoxyemodin) (1c)

A solution of emodin (27.0 g, 0.1 mol) in Ac_2O (500 mL) was refluxed for 3 h with a trace of conc. H_2SO_4 (0.5 mL). The reaction mixture was cooled to room temperature and poured onto crushed ice to afford 1,3,8-triacetoxyemodin as yellow needles (35.1 g, 88%): mp 196-198°C (from EtOAc) [lit. (Anslow et al., 1940): mp 193-195°C]. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 7.99 (d, J = 2.0 Hz, H4), 7.92 (d, J = 2.0 Hz, H5), 7.21 (d, J =

2.0 Hz, H2), 7.19 (d, J = 2.0 Hz, H7), 2.47 (s, 3H), 2.42 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H). ^{13}C-NMR ($CDCl_3$, 62.5 MHz) δ 181.4, 179.7, 169.5, 169.0, 167.9, 154.6, 151.6, 150.2, 146.4, 135.6, 134.0, 130.9, 126.0, 123.4, 123.2, 123.0, 118.3, 21.7, 21.1 (two C's), 21.0.

6,6-Dibromomethyl-1,3,8-triacetoxyanthracene-9,10-dione (6c)

Method A

A solution of **2c** (3.96 g, 0.01 mol), NBS (7.08 g, 0.04 mol), and benzoyl peroxide (300 mg) in a mixture of CCl_4 (150 mL) and dry benzene (500 mL) was refluxed for 24 h. The hot solution was filtered to remove the separated succinimide and then the filtrate was cooled in ice to give a pale yellow crystalline solid which was chromatographed on silica gel eluting with hexane: CH_2Cl_2 (1:3). **6-Bromomethyl-1,3,8-triacetoxyanthracene-9,10-dione** (**7**) (380 mg, 8%): mp 232-234°C. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 8.17 (d, J = 2.0 Hz, H5), 7.87 (d, J = 2.0 Hz, H4), 7.67 (d, J = 2.0 Hz, H7), 7.47 (d, J = 2.0 Hz, H2), 4.87 (s, 2H), 2.40 (s, 6H), 2.33 (s, 3H). **6,6-Dibromomethyl-1,3,8-triacetoxyanthracene-9,10-dione** (**6c**): Long needles (R_f = 0.2) (4.58 g, 83%): mp 178-179°C. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 8.29 (d, J = 2.0 Hz, H5), 7.95 (d, J = 2.3 Hz, H4), 7.64 (d, J = 2.0 Hz, H7), 7.25 (d, J = 2.3 Hz, H2), 6.64 (s, 1H), 2.44 (s, 3H), 2.42 (s, 3H), 2.34 (s, 3H). ^{13}C-NMR ($CDCl_3$, 62.5 MHz) δ 180.43, 179.08, 168.99, 168.90, 167.86, 154.94, 151.58, 150.62, 147.81, 135.32, 134.62, 128.63, 126.01, 123.71, 123.10, 122.96, 118.46, 37.31, 21.10, 21.02, 21.00. MS (ESI): m/z = 553 $[M + H]^+$.

Method B

A solution of **2c** (3.96 g, 0.01 mol), NBS (14.2 g, 0.08 mol), and benzoyl peroxide (300 mg) in a mixture of CCl_4 (150 mL) and dry benzene (500 mL) was refluxed for 24 h. The hot solution was filtered to remove the separated succinimide and then the filtrate was cooled in ice to give a pale yellow crystalline solid which was flash chromatographed on silica gel eluted with hexane:CH_2Cl_2 (1:3) to yield **6c** as long needles (hexane: CH_2Cl_2, 4.80 g, 87%): Spectral data were identical to those from Method A.

1,3,8-Triacetoxyanthracene-9,10-dione-6-carbaldehyde (3c)

A solution of $AgNO_3$ (0.50 g, 2.94 mmol) in distilled H_2O was slowly added to a refluxing solution of **6c** (83 mg, 0.15 mmol) in EtOH (20 mL). A precipitate, AgBr, formed immediately and was filtered off. The aqueous filtrate was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layers were dried over $MgSO_4$ and worked up as usual to give a semi-solid material which was chromatographed on silica gel eluted with CH_2Cl_2. The early fractions (R_f = 0.3) afforded 1,3,8-triacetoxyanthracene-9,10-dione-6-carbaldehyde (**3c**) (42 mg, 68%): mp 217-218°C. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 10.12 (s, 1H, CHO), 8.64 (d, 1H, J = 1.3 Hz, H5), 7.96 (d, 1H, J = 2.3 Hz, H4), 7.86 (d, 1H, J = 1.3 Hz, H7), 7.26 (d, 1H, J = 2.3 Hz, H2), 2.44 (s, 3H), 2.42 (s, 3H), 2.34 (s, 3H). ^{13}C-NMR ($CDCl_3$, 62.5 MHz) δ 189.44, 180.30, 179.37, 169.12, 168.95, 167.88, 155.10, 151.60, 150.92, 140.15, 135.24, 135.17, 129.06, 129.00, 126.94, 123.82, 123.18, 118.52, 21.07, 20.96 (two C's). MS (ESI): m/z = 411 $[M + H]^+$. The latter fractions (R_f = 0.1) afforded 1,8-diacetoxy-3-hydroxyanthracene-9,10-dione-6-carbaldehyde (**8**) (13.8 mg, 25%): mp 208°C. ^{1}H-NMR (DMSO-d_6, 250 MHz) δ 11.51 (br. s, 1H), 10.16 (s, 1H, CHO), 8.56 (d, 1H, J = 0.8 Hz, H5), 8.02 (d, 1H, J = 0.8 Hz, H7), 7.48 (d, 1H, J = 1.8 Hz, H4), 6.95 (d, 1H, J = 1.8 Hz, H2), 2.40 (s, 3H), 2.37 (s, 3H). ^{13}C-NMR ($CDCl_3$, 62.5 MHz) δ 191.67, 180.92, 178.91, 169.05, 168.86, 163.39, 152.40, 150.23, 139.84, 135.67, 135.03, 129.42, 128.66, 125.46, 117.65, 117.06, 111.24, 20.89, 20.86. MS (ESI): m/z = 369 $[M + H]^+$.

6-Hydroxymethyl-1,3,8-trihydroxyanthracene-9,10-dione (Citreorosein) (2a)

Method A

A mixture of **3c** (0.33 g, 0.8 mmol) and $NaBH_4$ (0.30 g) in CH_3OH (50 mL) was stirred for 4 h. The reaction mixture was made acidic with conc. HCl and concentrated to 50 mL of volume, to which additional H_2O (400 mL) was added. The precipitate formed was filtered, washed with H_2O, dried, and washed with $CHCl_3$ to yield **2a** (0.22 g, 95%) as orange-yellow needles (CH_3OH): mp 288-289°C [lit. (Anslow et al., 1940): mp 288°C]. ^{1}H-NMR (DMSO-d_6, 250 MHz) δ 12.08 (s, 1H, OH), 12.05 (s, 1H, OH), 11.42 (s, 1H, OH), 7.63 (d, 1H, J = 1.0 Hz, H5), 7.24 (d, 1H, J = 1.0 Hz, H7), 7.11 (d, 1H, J = 2.3 Hz, H4), 6.59 (d, 1H, J = 2.3 Hz, H2), 5.60 (br. s, 1H, CH_2-OH), 4.60 (s, 2H, CH_2-O). ^{13}C-NMR (DMSO-d_6, 62.5 MHz) δ 189.80 (C9), 181.43 (C10), 165.62 (C8), 164.52 (C6), 161.50 (C1), 152.92 (C3), 135.20 (C10a), 132.97 (C4a), 120.83 (C2), 117.12 (C4), 114.13 (C9a), 109.07 (C8a), 108.84 (C5), 107.98 (C7), 62.05. IR (KBr) υ 3394, 3064, 1628 cm^{-1}.

Method B

The same procedure described above for Method A was employed with **7** (184 mg, 0.5 mmol) and afforded **2a** (126 mg, 88%). Spectral data were identical to those from Method A.

Method C

The same procedure described above for Method A

was employed with **3a** which afforded **2a** in 98% yield. Spectral data were identical to those from Method A.

6-Formyl-1,3,8-trihydroxyanthracene-9,10-dione (3a)

Method A

A mixture of **3c** (82 mg, 0.20 mmol) in a mixture of 5% NaOH (20 mL) and dioxane (20 mL) was refluxed for 0.5 h and cooled to room temperature. The reaction mixture was made acidic with conc. HCl to afford precipitates which were filtered, washed with H_2O, dried, and washed with $CHCl_3$ to yield **3a** (48 mg, 83%) as orange-yellow needles: mp 280-281°C [lit. (Hauschild et al., 1971), mp 272-274°C]. ^{1}H-NMR (DMSO-d_6, 250 MHz) δ 12.08 (s, 1H, OH), 12.05 (s, 1H, OH), 10.09 (s, CHO), 8.03 (d, 1H, J = 1.0 Hz, H5), 7.74 (d, 1H, J = 2.3 Hz, H4), 7.11 (d, 1H, J = 2.3 Hz, H7), 6.57 (d, 1H, J = 2.3 Hz, H2), 5.60 (br. s, 1H, C3-OH). ^{13}C-NMR (DMSO-d_6, 62.5 MHz) δ 192.11 (HC=O), 189.01 (C9), 180.77 (C10), 166.36 (C8), 164.79 (C6), 161.45 (C1), 141.04 (C3), 134.98 (C10a), 133.98 (C4a), 124.39 (C2), 119.27 (C4), 118.01 (C9a), 109.37 (C8a), 109.22 (C5), 108.07 (C7).

Method B

A mixture of **8** (74 mg, 0.20 mmol) in 5% NaOH (20 mL) was refluxed for 0.5 h and cooled to room temperature. The reaction mixture was made acidic with conc. HCl to afford precipitates which were filtered, washed with H_2O, dried, and washed with $CHCl_3$ to yield **3a** (50 mg, 88%) as orange-yellow needles: mp 280-281°C (dec). Physical and spectral data were identical to those obtained from Method A.

6-Formyl-1,3,8-trihydroxyanthracene-9,10-dione dimethyl acetal (9)

A mixture of **3c** (82 mg, 0.20 mmol) in 5% NaOH in CH_3OH (20 mL) was refluxed for 0.5 h and cooled to room temperature. The reaction mixture was made acidic with conc. HCl to afford precipitates which were filtered, washed with H_2O, dried, and washed with $CHCl_3$ to yield **9** (57 mg, 87%) as orange-yellow needles (EtOAc): mp 219°C. ^{1}H-NMR (DMSO-d_6, 250 MHz) δ 12.02 (s, 1H, OH), 12.00 (s, 1H, OH), 10.09 (s, CHO), 7.62 (d, 1H, J = 1.0 Hz, H4), 7.24 (d, 1H, J = 1.0 Hz, H5), 7.08 (d, 1H, J = 2.3 Hz, H2), 6.56 (d, 1H, J = 2.3 Hz, H7), 5.46 (s, 1H), 3.30 (s, 6H). ^{13}C-NMR (DMSO-d_6, 62.5 MHz) δ 189.55 (C9), 180.08 (C10), 165.72 (C8), 164.54 (C6), 161.20 (C1), 146.98 (C3), 135.04 (C10a), 133.14 (C4a), 121.90 (C2), 117.22 (C4), 115.43 (C9a), 109.03 (C8a), 108.91 (C5), 107.94 (C7), 101.13, 52.94. MS (ESI): m/z = 331 $[M + H]^+$.

6-Methyl-1,3,8-trihydroxy-10*H*-anthracene-9-one (5a)

Method A

To a heated solution of **1a** (1.35 g, 5 mmol) in glacial HOAc (70 mL) at 100°C, $SnCl_2{\cdot}2H_2O$ (5.0 g, 22 mmol) in conc. HCl (33 mL) was added. The resulting reaction mixture was refluxed for 3 h and filtered. The filtrate was diluted with water (100 mL) to give the desired precipitates (1.15 g, 90%) in the form of yellow-green plates: mp 258°C (dec) [lit. (Jacobson and Adams, 1924): mp 250-258°C]. ^{1}H-NMR (DMSO-d_6, 250 MHz) δ 12.38 (s, 1H, OH), 12.21 (s, 1H, OH), 10.86 (s, 1H, OH), 6.76 (br. s, 1H, H5), 6.67 (s, 1H, H7), 6.41 (s, 1H, H2), 6.22 (s, 1H, H4), 4.29 (s, 2H), 2.31 (s, 3H). ^{13}C-NMR (DMSO-d_6, 62.5 MHz) δ 191.09, 164.98, 164.55, 161.69, 147.06, 144.96, 141.98, 119.88, 115.13, 112.82, 108.40, 107.38, 100.97, 32.24, 21.57.

Method B

To a heated solution of **3a** (0.14 g, 0.5 mmol) in glacial HOAc (15 mL) at 100°C, $SnCl_2{\cdot}2H_2O$ (417 mg, 2.2 mmol) in conc. HCl (3.3 mL) was added. The resulting reaction mixture was refluxed for 3 h and filtered. The filtrate was diluted with water (100 mL) to give the desired precipitates (0.12 g, 86%): mp 258-259°C (dec). Spectral data were identical to those obtained from the Method A.

6-Hydroxymethyl-1,3,8-trihydroxy-10*H*-anthracene-9-one (5b)

To a heated solution of **2a** (143 mg, 0.5 mmol) in glacial HOAc (15 mL) at 100°C was added $SnCl_2{\cdot}2H_2O$ (417 mg, 2.2 mmol) in conc. HCl (3.3 mL). The resulting reaction mixture was refluxed for 3 h and filtered. The filtrate was diluted with water (100 mL) to give orange-red needles as an acetate ester (6-hydroxymethyl-1,8-dihydroxy-10*H*-anthracen-3-yl)acetate) of the desired product: mp 229°C. ^{1}H-NMR (DMSO-d_6, 250 MHz) δ 12.30 (s, 1H, OH), 12.29 (s, 1H, OH), 10.85 (s, 1H, OH), 6.93 (s, 1H), 6.82 (s, 1H), 6.44 (d, 1H, J = 2.0 Hz), 6.24 (d, 1H, J = 2.0 Hz), 5.01 (s, 2H), 4.36 (s, 2H), 2.34 (s, 3H). ^{13}C-NMR (DMSO-d_6, 62.5 MHz) δ 190.96, 170.08, 165.14, 164.58, 161.60, 144.97, 144.57, 142.33, 117.22, 114.34, 113.05, 108.41, 107.36, 100.96, 64.40, 32.35, 20.56. Hydrolysis of this ester by refluxing 1 N NaOH followed by acidification with conc. HCl afforded orange-red solid in quantitative yield: mp 280-281°C [lit. (Cameron and Raverty, 1976): mp > 250°C]. Unreported spectral data are as follows: ^{1}H-NMR (DMSO-d_6, 250 MHz) δ 12.31 (s, 1H, OH), 12.05 (s, 1H, OH), 10.87 (s, 1H, OH), 7.67 (s, 1H), 7.26 (s, 1H), 7.18 (d, 1H, J = 2.4 Hz), 6.65 (d, 1H, J = 2.4 Hz), 4.61 (s, 2H), 3.36 (s, 2H). MS (ESI): m/z = 273 $[M + H]^+$.

Fluorometric μ-calpain assay

The assay was performed on a final volume of 100 μL in a microplate. A stock solution of pep1 with the compounds was prepared in DMSO and stored at −20 °C before use. μ-Calpain inhibition was assayed in a reaction buffer (50 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 1 mM EGTA and 5 mM β-mercaptoethanol, pH 7.5) with 100 μM pep1, 2.5 mM $CaCl_2$, and 5.25 units/mL μ-calpain. The reaction was initiated by adding in the order of substrate, μ-calpain, each compound, and $CaCl_2$ solution. Resulting mixture was incubated while shaken at room temperature for 30 min. The end-point fluorescence intensity in each well was measured in a Microplate Fluorescence Reader (SpectraMAX GEMINI EM, Molecular Devices) with 320 nm excitation and 420 nm emission wavelengths. The IC_{50} values were obtained using the data graphing software TableCurve 2D (Systat software Inc.). Fluorescence intensity was indicated in relative fluorescence units (RFU), calculated by subtracting the RFU of the no-enzyme control from all other values. The percentage of inhibition was expressed as the percentage of change in RFU, reflecting enzyme activity in the presence versus the absence (100% activity) of compounds.

RESULTS AND DISCUSSION

All attempts to oxidize the methyl groups of emodin (**1a**) and 1,3,8-tri-*O*-acetylemodin (**1c**) with conventional oxidizing agents such as chromium trioxide, ceric ammonium nitrate, and lead tetraacetate to the corresponding carbaldehydes or carboxylic acids afforded only unchanged starting compounds, as have been discussed for 1,3,8-tri-*O*-methylemodin (**1b**) previously (Lackner et al., 2005).

Although selenium oxide has long been used as a selective oxidizing reagent for the preparation of aromatic aldehydes under mild and one-pot conditions (Mlochowski et al., 1965; Chandler et al., 1981; Zhang et al., 2008), all of our attempts to prepare emodin carbaldehyde and related aldehydes by SeO_2 oxidation failed. Reaction of **1a-c** with SeO_2 instead resulted in unchanged starting materials as the only products.

Although a two-step conversion of **1b** to the corresponding 1,3,8-trimethoxyemodin carbaldehyde (**5b**) by Salama et al. (Salama et al., 2003) has been reported as an efficient method, such attempts have never been applied to emodin (**1a**) and 1,3,8-tri-*O*-acetyl emodin (**1c**) as yet. In addition, the demethylation of 1,3,8-trimethoxyemodin carbaldehyde by conventional methods to emodin carbaldehyde (**3a**) resulted in a messy mixture of products, thus leading to a low yield after tedious column chromatography. Such impracticability definitely requires a new feasible synthesis procedure for emodin carbaldehyde. In fact, the reaction of **1a** with 4 equivalents of *N*-bromosuccinimide (NBS) in the presence of benzoyl peroxide did not produce the corresponding 3,3-dibromomethyl derivative (**6a**). However, a reaction of **1c** with 4 equivalents of NBS afforded the 3,3-dibromomethyl derivative (**6c**) and its mono-brominated congener (**7**) in 83% and 8% yields respectively, while 8 equivalents of NBS afforded **6a** as the only product in a 87% yield. Subsequent Ag(I)-mediated hydrolysis of dibromide (**6c**) in aq. ethanol afforded **3c** and **8** in a ratio of 1:1.2, respectively. Attempts for optimizing hydrolytic conditions for **6c** to lead to **3c** as an only product failed, but a longer reaction time (over 12 h) led to an increase of the portion of **8**, to over 99%. Subsequent hydrolyses of **3c** and **8** in aq. dioxane afforded **3a** in 89% and 94% yields, respectively, while hydrolysis in aq. CH_3OH afforded the corresponding dimethyl acetal (**9**) in a 87% yield with a trace of **3a** (<1%). Finally the reduction of **3c** and **8** by $NaBH_4$ afforded citreorosein (**2a**) in 95% and 88% yields, respectively. Similarly, reduction of **3a** by $NaBH_4$ afforded **2a** in a quantitative yield.

Each ^1H and ^{13}C resonance was assigned by double-quantum filtered COSY experiments and NOE for the selected protons, as well as by comparison of reported values of emodin (Tamano and Koketsu, 1982; Danielson et al., 1992) and citreorosein (Fujimoto et al., 2004). Comparison of the ^1H- and ^{13}C-NMR spectra of **8** with those of **3c** indicated that the C3 resonance (δ 163.39) of **8** was down-field shifted by 8.62 ppm compared to that of **3c** (δ 154.77) as referenced to the acetylation shift rule (Terui et al., 1976; Ishii et al., 1977). Proton resonance at δ 2.40 and carbon resonance at δ 168.51 of the acetyl group at C3-OH were absent in **8**. In addition, a proton resonance at δ 11.51 (br. s) for C3-OH of **8** was well matched to that of emodin at δ 10.42 (br. s), of which the IR spectrum showed a strong OH stretching band at 3136-3066 cm^{-1}.

Although emodin anthrone (**5a**) was additionally prepared on a few occasions (Brockmann et al., 1957; Cameron and Raverty, 1976; Falk and Schoppel, 1991), **5b** and **5c** have not been reported as yet. Among the conventional synthesis methods converting anthraquinones to anthrones a method employing stannous chloride in a 1:2 mixture of glacial HOAc and conc. HCl was claimed to be the most efficient (Haller and Goodall, 1924). Such a reduction method was applied to **1a** and **2a** to give the corresponding anthrone compounds **5a** and **5b** in 90% and 92% yields, respectively. Reduction of **3a** resulted in **5a** instead of **5c**, however, oxidation of **5b** with MnO_2 afforded **5c** in a

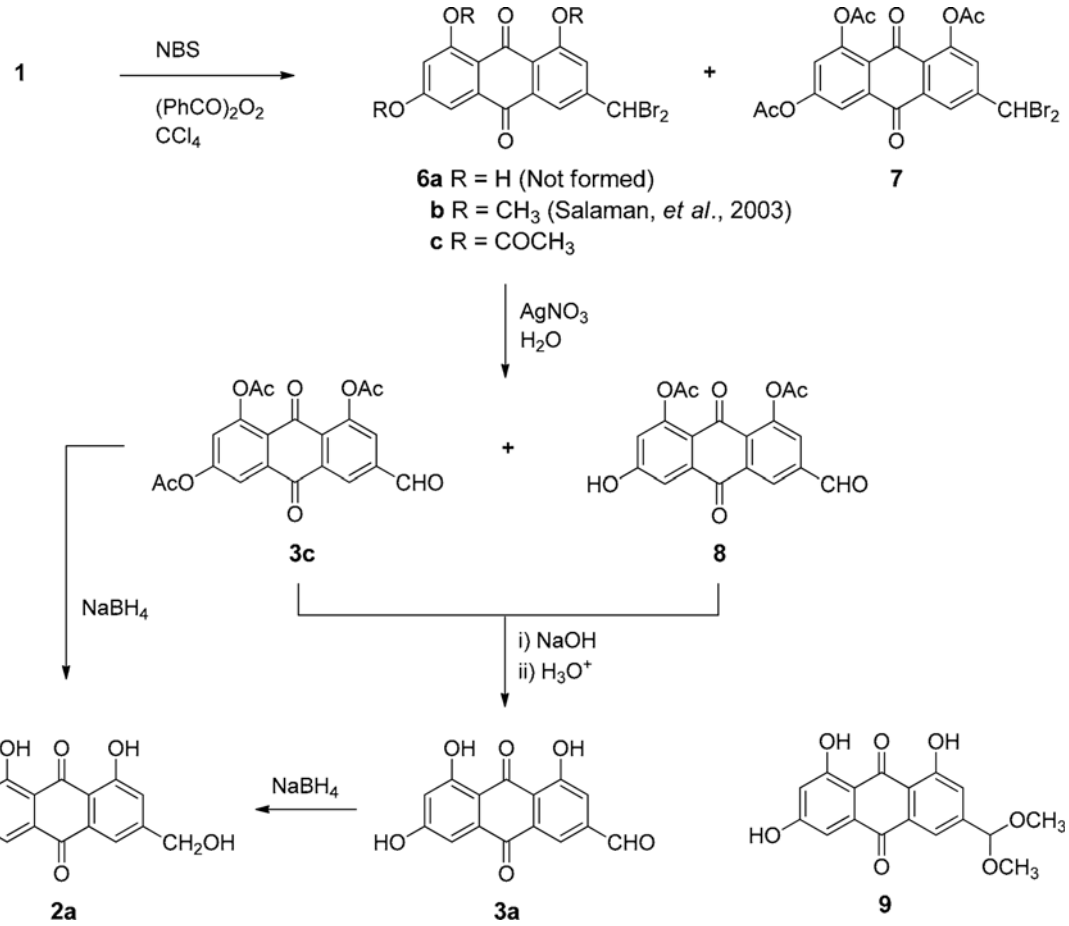


Fig. 2. Synthesis of emodin derivatives

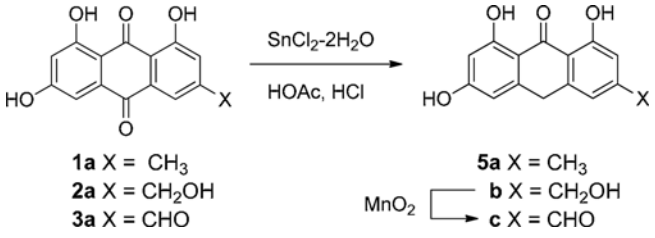


Fig. 3. Synthesis of 10-deoxyemodins

86% yield.

Inhibitory activities of the compounds prepared against μ-calpain were evaluated by employing the method reported previously (Kang et al., 2009) and are summarized in Table I. Known calpain inhibitors, MDL28170 [benzyl {3-methyl-1-oxo-1-[(1-oxo-3-phenylpropan-2-yl)amino]butan-2-yl}carbamate] and E64d {(+)-2*S*,3*S*-*trans*-[(*S*)-3-methyl-1-(3-methylbutylcarbamoyl) butylcarbamoyl]-2-oxiranecarboxylic acid}, were used as positive controls. All the compounds, with the exception of **5a**, strongly inhibited μ-calpain and had IC_{50} values in the range of 20.15 ± 0.81 to 78.76 ± 0.05 μM. 10-Desoxycitreorosein (**5b**) and emodin carbaldehyde (**3a**) inhibited μ-calpain the most and their activities were 3.1 and 2.4 times more potent than that of E64d, respectively.

Table I. The inhibitory activities of compounds prepared against μ-calpain

Compound	Inhibitory activity [IC_{50} (μM)[a]]	Compound	Inhibitory activity [IC_{50} (μM)[a]]
1c	N/T	**5c**	N/T
2a	78.76 ± 0.05	**6c**	49.49 ± 0.66
3a	25.77 ± 0.32	**8**	50.52 ± 0.24
3c	56.07 ± 0.05	**9**	49.41 ± 0.08
5a	102.53 ± 2.04	MDL28170	0.1143 ± 0.0034
5b	20.15 ± 0.81	E64d	62.26 ± 7.73

[a]Each data point represents mean ± S.D. from three different experiments performed in triplicate.

In conclusion, an efficient and practical synthesis procedure for the preparation of emodin carbaldehyde and citreorosein was established from emodin, in which ω,ω'-dibromomethylemodin triacetate was prepared as a key intermediate by NBS-mediated bro-

mination of 1,3,8-triacetylemodin. Reduction of emodin and citreorosein with $SnCl_2$ in a 1:1 mixture of HOAc and HCl afforded the corresponding anthrones in 90% and 92% yield, respectively, while the corresponding 10-desoxyemodin carbaldehyde was prepared by MnO_2 oxidation of 10-desoxycitreorosein. 10-Desoxycitreorosein and emodin carbaldehyde showed inhibitory activities against μ-calpain at the level of IC_{50} 20.15 and 25.77 μM, respectively. Studies on the derivatization and biological properties of the compounds are in progress.

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