Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supporting Information

Synthesis of Rhein and Diacerein: A Chemoenzymatic Approach using Anthrol Reductase of *Talaromyces islandicus*

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I. General Remarks

All commercial reagents were obtained from Sigma-Aldrich Chemical Co. and Sisco Research Laboratories, India. Emodin was isolated from commercially available *Rheum emodi* plant extract using the reported procedure.[1] Emodic acid was synthesized by using a published procedure.[2] Reactions were monitored by thin-layer chromatography (TLC, 0.25 mm E. Merck silica gel plates, $60F_{254}$) and the plates were visualized by using UV light. Column chromatography was performed on silica gel 60–120/230–400 mesh obtained from S. D. Fine Chemical Co., India. Yields refer to chromatographically pure materials; conversions were calculated from the ^{1}H NMR spectra of the crude products. ^{1}H NMR spectra were recorded on Bruker 400 Ultra Shield instruments using deuterated solvents. Proton coupling constants (*J*) are reported as absolute values in Hz. ^{13}C NMR spectra were recorded on Bruker 400 Ultra Shield instruments operating at 100 MHz. Chemical shifts (δ) of the ^{1}H and ^{13}C NMR spectra are reported in ppm with a solvent resonance as an internal standard. For ^{1}H NMR: chloroform-d_1 7.26, acetone-d_6 2.05, DMSO-d_6 2.50; for ^{13}C NMR: chloroform-d_1 77.16, acetone-d_6 29.84, DMSO-d_6 39.52. The following abbreviations were used to explain the multiplicities: s = singlet, brs = broad singlet, d = doublet, dd = doublet of a doublet, ddd = doublet of a doublet of doublet, t = triplet, dt = doublet of a triplet, m = multiplet. Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies). For determination of the enantiomeric excess (ee) the chiral phases Chiralcel OD-H (Daicel Inc., 250 × 4.6 mm, 5 µm), Chiralcel OZ-H (Daicel Inc., 250 × 4.6 mm, 5 µm) were used on Agilent Technologies 1260 Infinity HPLC system equipped with OpenLAB CDS v2.3 software.

II. Protein sequence

> *ARti-2* (Anthrol reductase)[3]

Organism: *Talaromyces islandicus* WF-38-12

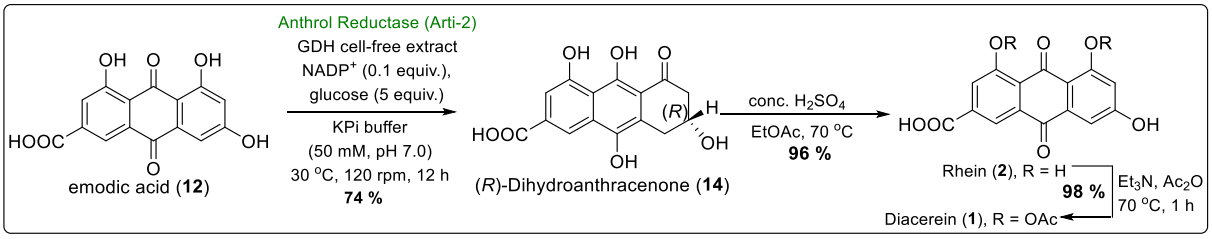
Codon-optimized Protein sequence :

MGHHHHHHHHHHSSGHIDDDDKHMMAIQEYIPGRLDGKVALVTGSGRGIGAAIAIQLGQLGAKVVVN
YSASATHAEKIVAEIKANGSDAIALKADVRQVFQTAKLFDDAVAHFGKLDVAVSNAGVVSFGHLKDV
TEEEFDRVFSLNTRGQFFVAREAYRVLGEGGRIILTSSNTSRDFSVPKHSLYSASKGAIDSFVRILSKDCG
DKKITINAVAPGGTVTDMFHDVSHHYIPNGEKYTPEERMQMAAHASPLHRNGFPQDIANVVGFLVSKE
AEWVNGKTLTLDGGAA

Cloning, Expression and Purification of Enzymes

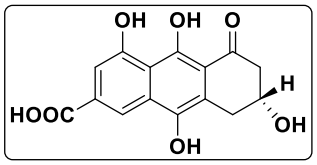
The required plasmid ARti_2 was transformed to competent *E. coli* BL21 (DE3) cells by applying a heat shock at 42 °C for 45 s. The transformed cells were grown overnight on a SOB-agar medium containing 100 µg/mL ampicillin. Single colony of required plasmid was picked and dispersed in 5 mL of LB-media (Lennox) supplemented with ampicillin (100 $\mu g \cdot mL^{-1}$) as required, followed by incubation overnight (37 °C, 220 rpm). The overnight cultures were diluted to 500 mL of medium each (ampicillin 100 $\mu g \cdot mL^{-1}$) and incubated at 37 °C, 160 rpm. IPTG (0.2 mM) was added to the mid-log phase (OD_{600} = 0.6) was reached, and cultures were incubated for 20 h at 18 °C, 160 rpm. The cells were then harvested by centrifugation (4 °C, 2,831 rcf, 20 min) and washed with potassium phosphate buffer (100 mM, pH 7.0). The harvested *E. coli* cells were resuspended in lysis buffer (20 mM Tris-HCl, pH 8.0; 2.5 mL per harvested cells of 500 mL medium) and lysis performed by by sonication (8 × 10 sec, Vibra-Cell Processors, model number VCX500, Sonics), followed by centrifugation (30 min, 12000×*g*, 4 °C). Glycerol (20% v/v) was added, and the crude enzyme preparation was frozen at –20 °C. The resultant clarified lysate was purified by Ni-NTA affinity chromatography. Non-specifically bound proteins were washed off with 5 mM and 20 mM imidazole in Tris buffer (25 mM Tris-HCl, pH 8.0). Elution was performed with 25 mM Tris buffer (pH = 8.0) containing 50and 250 mM imidazole. The purity of the enzyme was confirmed by SDS-PAGE. The fractions containing purified proteins are collected and desalted by gel filtration (Econo-Pac 10DG desalting gel column, Bio-Rad). The concentration of the protein was performed by ultrafiltration (Vivaspin 20R centrifugal filter units, 10 kDa nominal molecular weight limit, Sartorius). The concentration of the protein was determined by measuring the UV absorption at 280 nm (NanoVue, GE Healthcare; extinction coefficient 16055 $M^{-1} \cdot cm^{-1}$, molecular weight 30.95 kDa).

III. Synthesis of Rhein & Diacerein via chemoenzymatic reduction of emodic acid (12)



Scheme S1. Synthesis of Rhein & Diacerein via chemoenzymatic reduction of emodic acid.

(*R*)-4,7,9,10-tetrahydroxy-5-oxo-5,6,7,8-tetrahydroanthracene-2-carboxylic acid (14)



$C_{15}H_{12}O_7$: 304.25 g/mol

To a 100 mL round bottom flask, $NADP^+$ (0.016 mmol, 0.1 equivalent), glucose (0.820 mmol, 5 equivalent) and GDH (82 U) was added into degassed buffer (50 mL, 50 mM KPi, pH 7.0, 1 mM EDTA, 1 mM DTT) at room temperature. After that emodic acid (**12**) (0.164 mmol in dmso, 5% w/v) was added followed by $Na_2S_2O_4$ (3.28 mmol, 20 equivalent). ARti-2_his (20.0 μM) was added to the reaction mixture and kept stirring (150 rpm) for 12 h at room temperature. Then ethyl acetate (3 X 10 mL) the reaction mixtures were subjected to vortex and centrifugation to completely separate the organic and aqueous layers. The combined organic layer was dried over anhydrous Na_2SO_4, evaporated to dryness at the rotary evaporator crude product was purified using column chromatography (silica gel 230-400 mess size, CH_2Cl_2: MeOH 1:19) to afford pure reduced anthraquinone (*R*)-**14**, Configuration was determined by chiral-HPLC.

Yield: 74%

TLC (MeOH: $CHCl_3$, 1:4 v/v): R_f = 0.19.

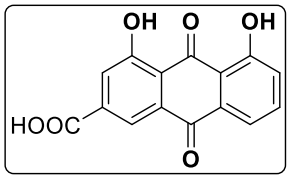
^{1}H NMR (400 MHz, dmso-d_6): δ (ppm) 2.74 (dd, *J* = 16.9, 6.6 Hz, 1H), 2.96 – 3.15 (m, 2H), 3.15 (dd, *J* = 16.7, 3.5 Hz, 1H), 4.27 – 4.33 (m, 1H), 5.21 (s, 1H), 7.22 (d, *J* = 1.5 Hz, 1H), 8.26 (d, *J* = 1.5 Hz, 1H), 9.97 (s, 1H), 15.06 (s, 1H).

^{13}C NMR (100 MHz, dmso-d_6): δ (ppm) 31.8, 46.3, 64.1, 109.4, 111.2, 114.3, 115.0, 119.1, 132.2, 132.5, 141.8, 156.5, 157.4, 167.1, 205.4.

HPLC [Flow rate: 1.2 mL/min; Typical injection volume: 10 µL; Isocratic: 85% n-Hexane, 15% Isopropanol; DAD: 280 nm (bandwidth = 4nm); Column: Chiralcel OZ-H, 5 µm, 4.6 mm (ϕ) x 250 mm (L), Temperature: 25 °C.]: Retention time (R_t), (*R*-**14**) = 21.56 min.; >99% ee (determined by comparison to rac-**14**, R_t (*S*-**14**) = 17.05 min, R_t (*R*-**14**) = 22.03 min).

Exact Mass [M+H]$^+$: 305.0656 (calculated), 305.0675 (found).

4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Rhein) (2):



$C_{15}H_8O_6$: 284.22 g/mol

(*R*)-3,8,9,10-tetrahydroxy-3,4-dihydroanthracen-1(2*H*)-one (**13**) (0.22 mmol) was dissolved in ethyl acetate (20 mL) and five drops of conc. H_2SO_4 was added into the reaction mixture and it refluxed at 70 ^oC for 12h. The reaction was monitor by checking TLC, after full consumption of the starting material, the reaction mixture was cooled to room temperature, evaporate the solvent at reduced pressure and subjected to purify using column chromatography as 5% CH_2Cl_2 in methanol as eluent to isolate yellow solid as only one component in 96% yield. The product was characterized by NMR spectroscopy and Mass spectrometry.

TLC (MeOH: CH_2Cl_2, 3:17 v/v): R_f = 0.24.

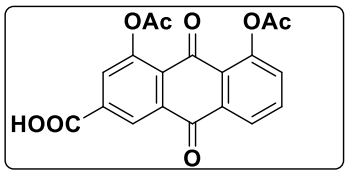
Yield = 96 %

^{1}H NMR (400 MHz, dmso-*d*$_6$): δ (ppm) 7.39 (d, *J* = 8.3 Hz, 1H), 7.71 (d, *J* = 7.25 Hz, 1H), 7.74 (s, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 11.91 (s, 1H).

^{13}C NMR (100 MHz, dmso-*d*$_6$): δ (ppm) 116.2, 118.6, 118.8, 119.4, 124.1, 124.6, 133.2, 133.8, 137.6, 138.5, 161.1, 161.4, 165.5, 181.0, 191.3.

Exact Mass [M+H]$^+$: 285.0399 (calculated), 285.0392 (found).

4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Diacerein) (1):



$C_{19}H_{12}O_8$: 368.30 g/mol

Compound **2** (0.087 mmol) was dissolved in triethylamine (500 μL) and acetic anhydride (1 ml). Then, the reaction mixture was stirred at 70 ^oC for 2 h and the reaction was monitored by TLC. After consumption of the whole starting material, the reaction mixture was cooled to room temperature and poured onto crushed ice to afford diacerein (**1**), in 98 % as a pale-yellow solid as crystal after filtration. The product was characterized by NMR spectroscopy and Mass spectrometry.

TLC (MeOH: CH_2Cl_2, 1:20 v/v): R_f = 0.24.

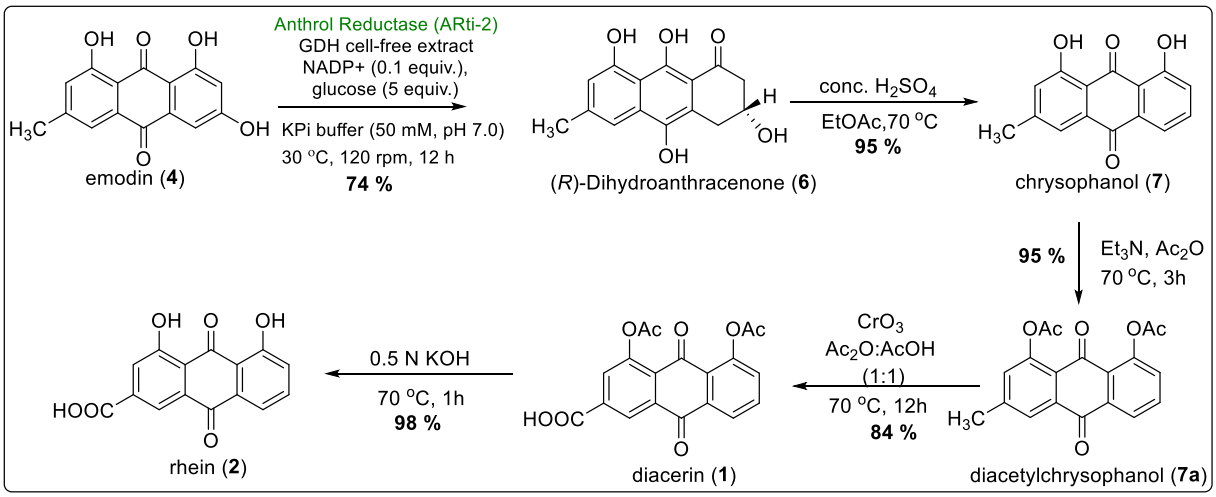
Yield = 98 %

^{1}H NMR (400 MHz, dmso-d_6): δ (ppm): δ 2.39 (s, 3H), 2.40 (s, 3H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.95 (t, *J* = 7.9 Hz, 1H), 8.03 (s, 1H), 8.15 (d, *J* = 7.7 Hz, 1H), 8.55 (s, 1H), 13.92 (brs, 1H).

^{13}C NMR (100 MHz, dmso-d_6): δ 20.8, 124.9, 125.0, 125.3, 128.0, 130.2, 130.6, 134.1, 134.6, 135.5, 136.4, 149.5, 149.7, 165.0, 169.0, 169.0, 180.3, 180.8.

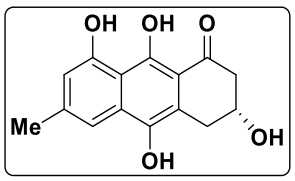
Exact Mass $[M+Na]^+$: 391.0424 (calculated), 391.0425 (found).

IV. Synthesis of diacerein (1) & rhein (2) via chemoenzymatic reduction of emodin



Scheme S2. Synthesis of diacerein (**1**) & rhein (**2**) via chemoenzymatic reduction of emodin.

(*R*)-3,8,9,10-tetrahydroxy-6-methyl-3,4-dihydroanthracen-1(2*H*)-one (6)[2]

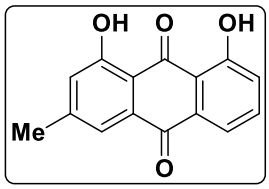


$C_{15}H_{14}O_6$: 274.27 g/mol

Reduction of emodin (**4**) was done as previously reported by our group.[2] To a 25 mL round bottom flask, $NADP^+$ (0.0018 mmol, 0.1 equivalent), glucose (0.185 mmol, 5 equivalent) and GDH (30 U) was added into degassed buffer (10 mL, 50 mM KPi, pH 7.0) at room temperature. After that emodin (**4**) (0.037 mmol in dmso, 5% w/v) was added followed by $Na_2S_2O_4$ (0.74 mmol, 20 equivalent). ARti-2_his (5.0 μM) was added to the reaction mixture and kept stirring (150 rpm) for 12 h at room temperature. Then ethyl acetate (3 X 10 mL) the reaction mixtures were subjected to vortex and centrifugation to completely separate the organic and aqueous layers. The combined organic layer was dried over anhydrous Na_2SO_4, after that, the crude product was purified using column chromatography to afford pure reduced emodin (**6**). All the analytical data were matched with the previously published report.[2]

Yield: 74%

1,8-dihydroxy-3-methylanthracene-9,10-dione (chrysophanol) (7):



$C_{15}H_{10}O_4$: 254.241 g/mol

Compound **6** (0.22 mmol) was dissolved in ethyl acetate (20 mL) and five drops of conc. H_2SO_4 was added into the reaction mixture and it refluxed at 70 ^oC for 12h. The reaction was monitor by checking TLC, after full consumption of the starting material, the reaction mixture was cooled to room temperature, evaporate the solvent at reduced pressure and subjected to purify using column chromatography as 30% ethyl acetate in hexane as eluent to isolate yellow solid as only one component in 95 % yield. The product was characterized by NMR spectroscopy and Mass spectrometry.

TLC (hexane: ethylacetate, 7: 3 v/v): R_f = 0.58.

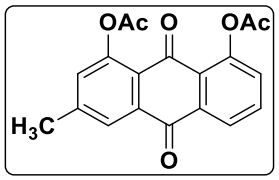
Yield: 95 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.46 (s, 3H), 7.08 (s,1H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.65 (m, 2H), 7.80 (d, *J*= 7.5 Hz, 1H), 11.99 (s, 1H), 12.10 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 22.4, 113.9, 116.0, 120.1, 121.5, 124.5, 124.7, 133.4, 133.8, 137.1, 149.5, 162.6, 162.9, 182.1, 192.7.

Exact Mass $[M+H]^+$: 255.0657 (calculated), 255.0654 (found).

3-methyl-9,10-dioxo-9,10-dihydroanthracene-1,8-diyl diacetate (7a):



$C_{19}H_{14}O_6$: 338.32 g/mol

Chrysophanol (**7**) (0.393 mmol) was dissolved in a solution of triethylamine (2.0 mL) and acetic anhydride (4 mL). Then, the reaction mixture was heated at 70 ^oC for 3h. The reaction was monitored by TLC. After completion, the reaction mixture was cooled to room

temperature, extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on rotary evaporator and purified by the column chromatography (silica gel 100-200 mess size, MeOH : CH_2Cl_2,1:19) to afford 3-methyl-9,10-dioxo-9,10-dihydroanthracene-1,8-diyl diacetate (**7a**) in 95 % yield.

TLC (EA: Hex, 2: 8 v/v): R_f = 0.27.

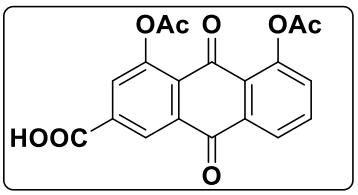
Yield: 95 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.43 (s, 3H), 2.44 (s, 3H), 2.45 (s, 3H), 7.21 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.74 (t, *J* = 8 Hz, 1H), 8.01 (s, 1H), 8.21 (d, *J* = 8 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 21.2, 21.8, 123.3, 125.5, 125.7, 126.0, 130.3, 130.8, 134.3, 134.6, 134.6, 146.5, 150.1, 150.2, 169.6, 169.6, 180.7, 182.3.

Exact Mass $[M+H]^+$: 339.0863 (calculated), 339.0887 (found).

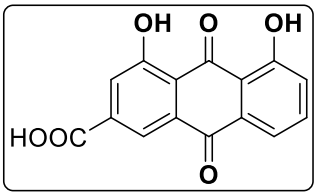
4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Diacerein) (1):



$C_{19}H_{12}O_8$: 368.30 g/mol

7a (3.53 mmol) was dissolved in a mixture of acetic acid (30.0 mL) and acetic anhydride (50.0 mL). Then, the solution of chromium trioxide (3.53 g, 35.3 mmol, 10.0 equiv.) dissolved, in acetic acid (20.0 mL) was gradually added to the solution of **7a** and the reaction mixture was stirred at 70 ^oC for 24 h. Then, the reaction mixture was cooled to room temperature, extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on a rotary evaporator and purified by the column chromatography (silica gel 230-400 mess size, CH_2Cl_2: MeOH 1:19) to afforded diacerein **1** in 84% yield.

4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Rhein) (2):



$C_{15}H_8O_6$: 284.22 g/mol

Diacerein (**1**) (0.054 mmol) was dissolved in 0.5(N) KOH solution (2 mL) and the reaction mixture was stirred at 70 ^oC for 1 h. After completion of the reaction as monitored through TLC, acidification was done with 10 % HCl solution and the reaction mixture was extracted with ethyl acetate (3 X 5 mL), washed with brine solution and dried over anhydrous sodium sulphate. The extracted organic layer was concentrated on a rotary evaporator and purified by the column chromatography (silica gel, CH_2Cl_2: MeOH 1: 19) to afforded rhein (**2**) in 98 % yield.

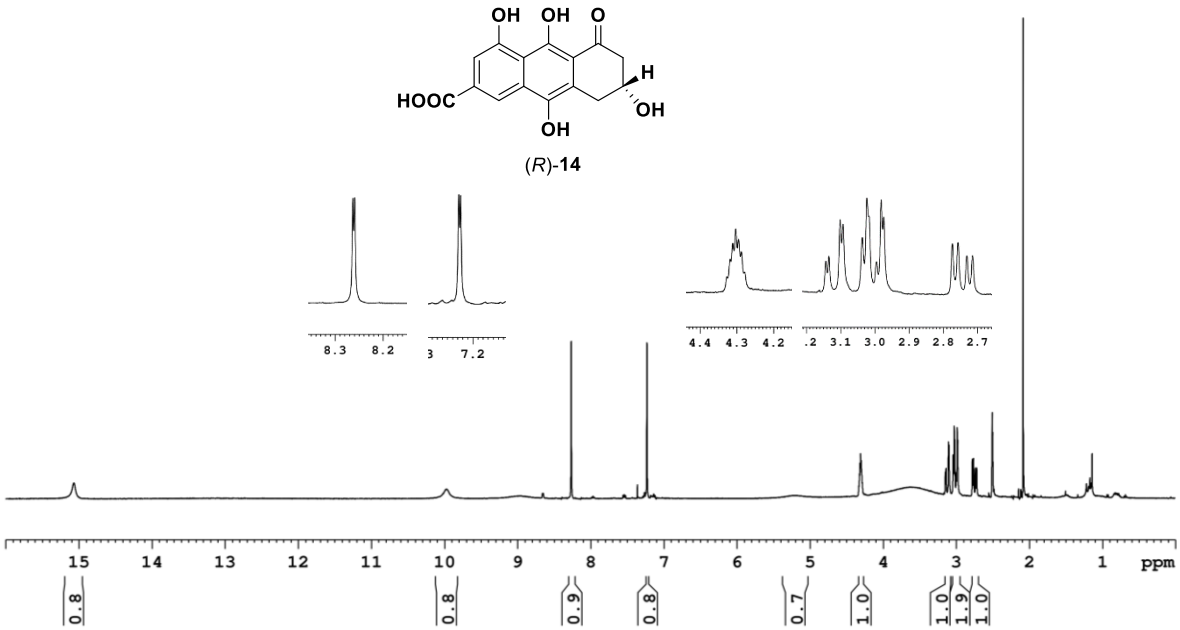
TLC (MeOH:CH_2Cl_2, 3:17 v/v): R_f = 0.24.

V. General procedure for the synthesis of racemic dihydroanthracenones (*rac*-14)[2]:

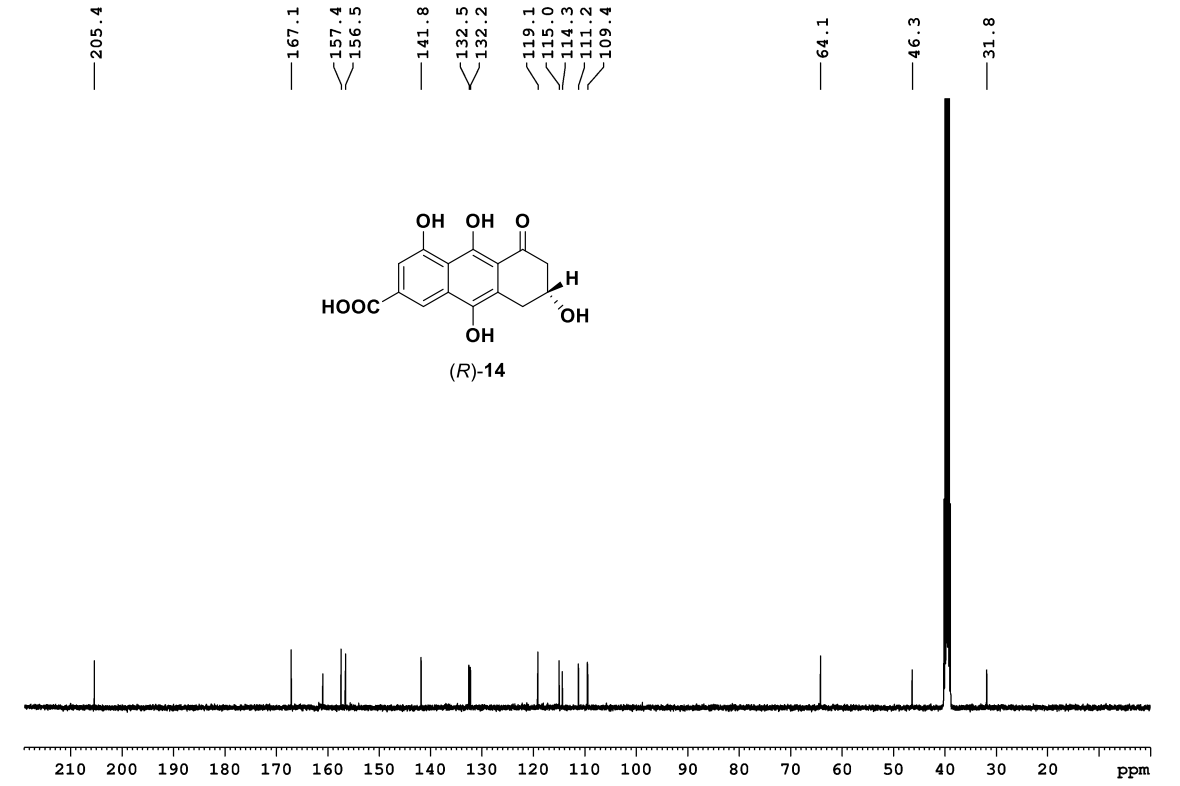
To a 50 ml round bottom flask in 10 mL degassed water the anthraquinone (**12**) (74 μmol) in methanol (10% v/v) was added in an argon atmosphere. $Na_2S_2O_4$ (20 equiv., 1.48 mmol) and $NaBH_4$ (20 equiv.,1.48mmol) were added portion-wise at ice-cold temperature. After 20 min, 1 N HCl was added and the reaction mixture was extracted in EtOAc and concentrated on a rotary evaporator. The crude reaction mixture was subjected to column chromatography and the product was purified using column chromatography.

NMR Spectra

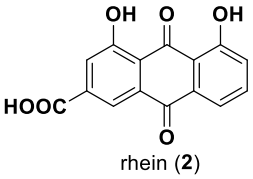
^{1}H NMR (400 MHz, dmso-d_6)



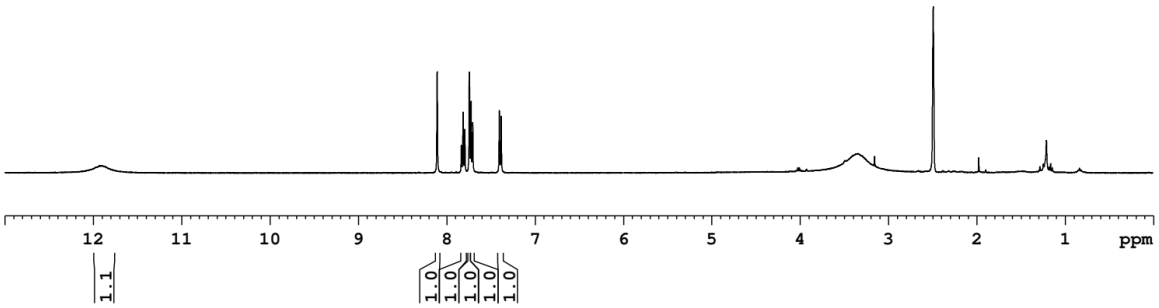
^{13}C NMR (100 MHz, dmso-d_6)



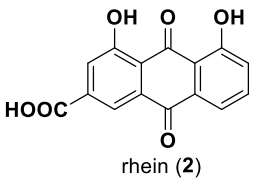
^{1}H NMR (400 MHz, dmso-d_6)



rhein (**2**)

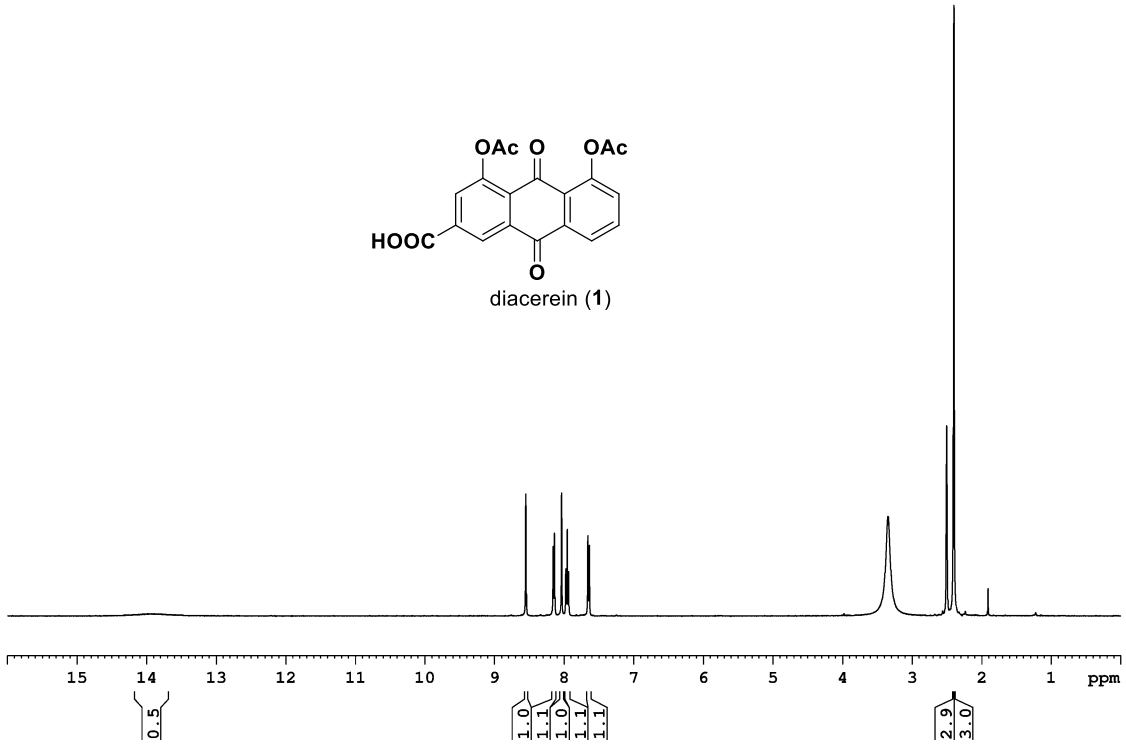


^{13}C NMR (100 MHz, dmso-d_6)

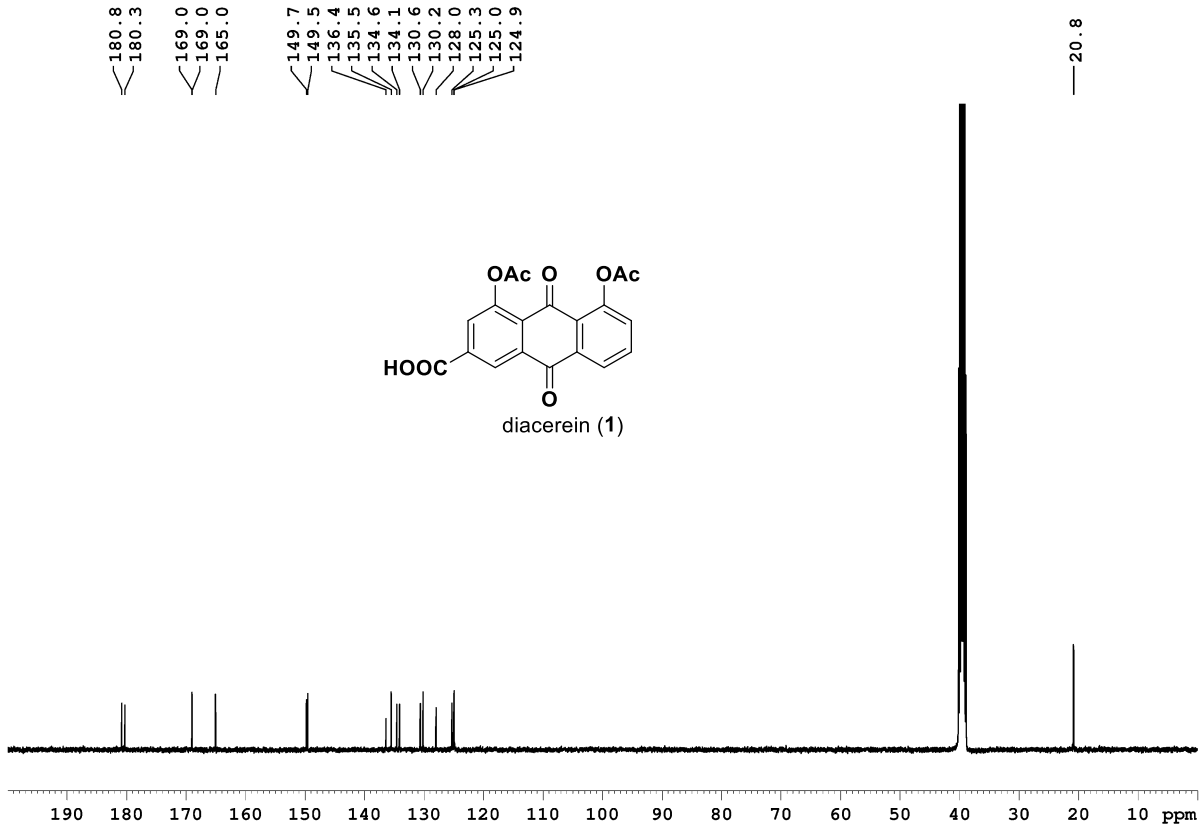


rhein (**2**)

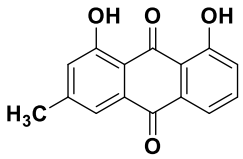
^{1}H NMR (400 MHz, dmso-d_6)



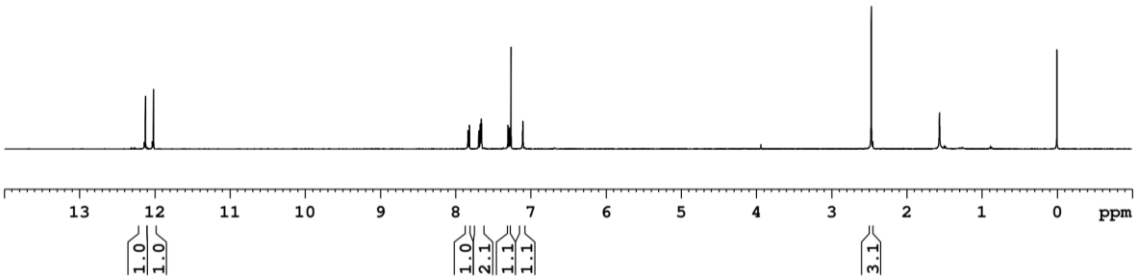
^{13}C NMR (100 MHz, dmso-d_6)



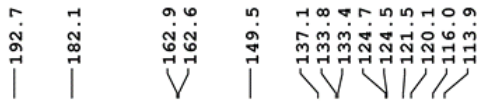
^{1}H NMR (400 MHz, $CDCl_3$)

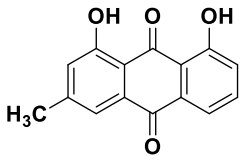


chrysophanol (**7**)



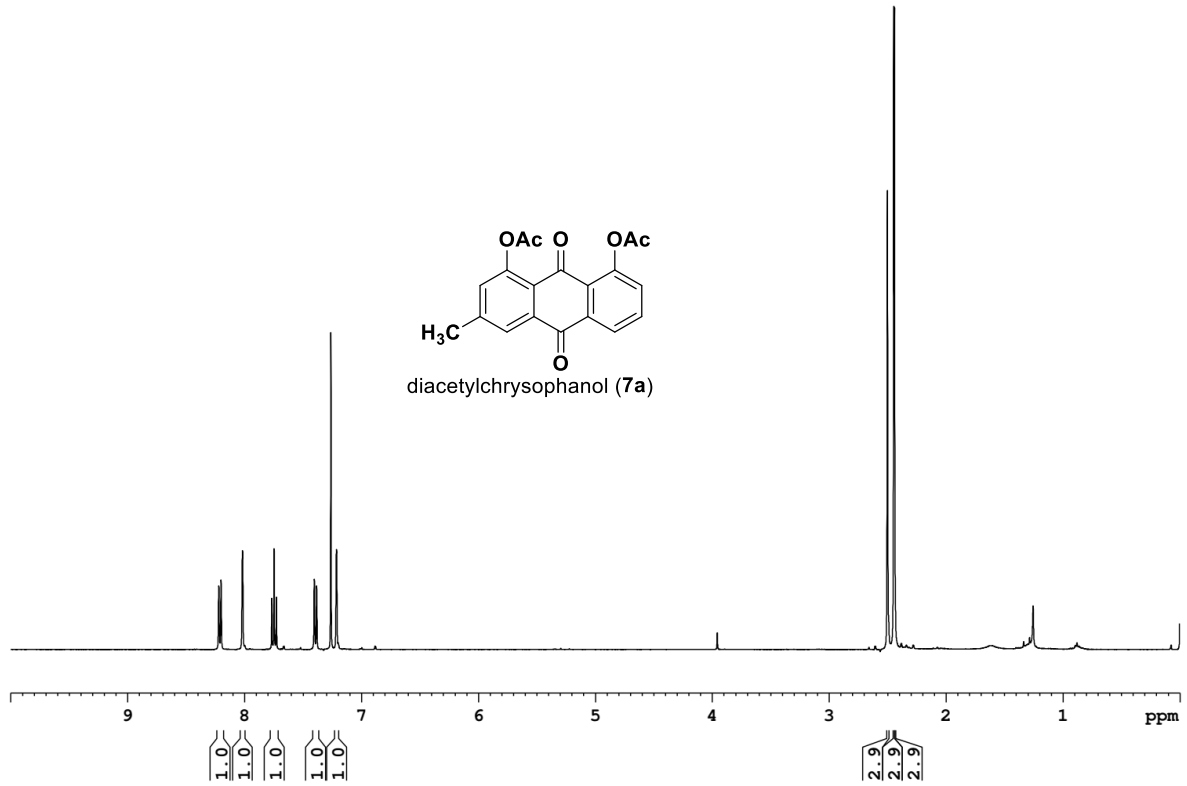
^{13}C NMR (100 MHz, $CDCl_3$)



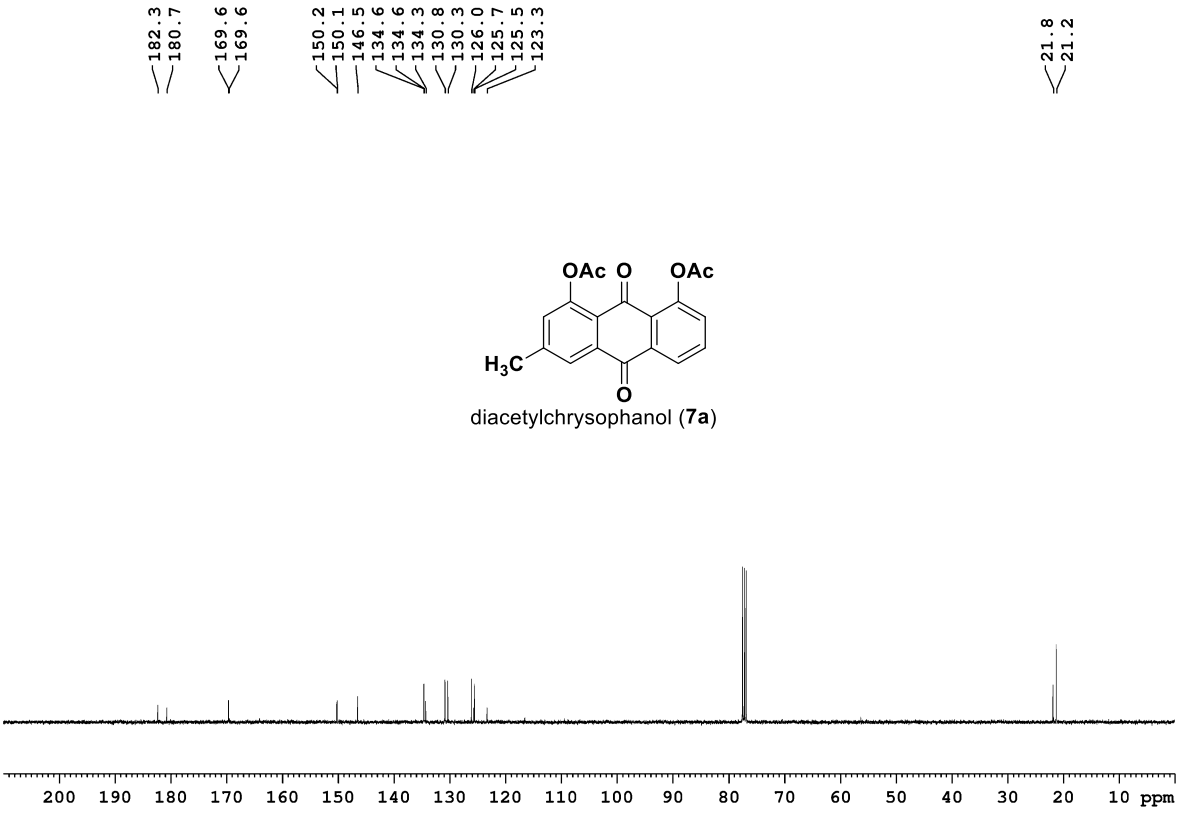


chrysophanol (**7**)

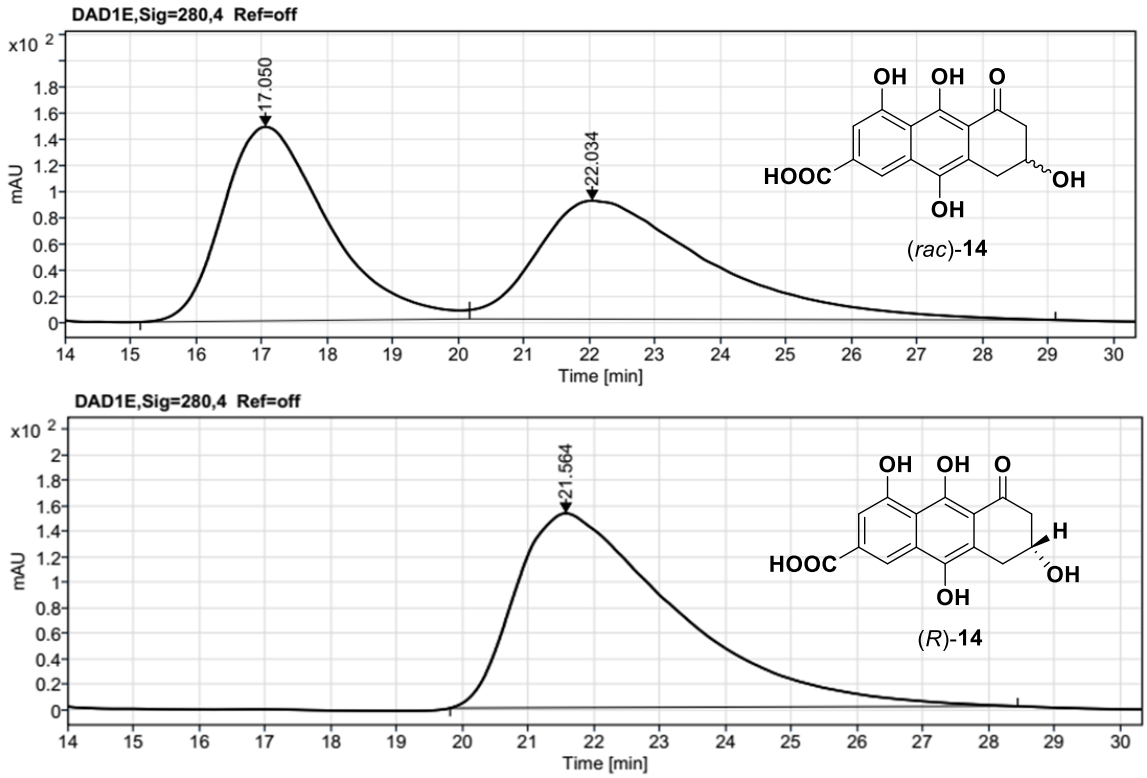
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100 MHz, $CDCl_3$)



VI. HPLC Chromatogram



VIII. References

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3. S. K. Singh, A. Rajput, A. De, T. Chakraborti and S. M. Husain, *Catal. Sci. Technol.*, ***2021***, *11*, *474–478.*