3,6-dimethoxyxanthone from 2,2',4,4'-tetrahydroxy-benzophenone via Microwave-Assisted Annulation

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Xanthones are tricyclic aromatic compounds that have multiple pharmacological uses due to their anti-tumor, antioxidant, anti-inflammatory, anti-bacterial, and potentially chemopreventive properties. The target of this research was to optimize a two-step synthesis of 3,6-dimethoxyxanthone (3) from 2,2',4,4'-tetrahydroxy-benzophenone (1) via microwave-assisted (200 °C, 30-40 min., 150 W) sodium acetate-catalyzed annulation. The product, 3,6-dihydroxyxanthone (2), was then methylated to (3) using dimethyl sulfate (DMS) and sodium carbonate in acetone at reflux. The product yields were 93% (>99% purity) for (2) and 94% (>99% purity) for (3). Characterization was accomplished using ^{1}H NMR, FTIR, melting point, TLC, HPLC, and GCMS. The product (3) was made available for additional screening and research, such as synthesizing dyes like fluorescein and its derivatives.

Keywords: xanthone, tetrahydroxybenzophenone, microwave annulation, dimethyl sulfate

Introduction

Xanthones are a class of molecules characterized by a highly stable tricyclic aromatic structure and are commonly found in nature.[1,2,3,4,5,6,7,8,9,10] Figure 1 shows the xanthone structure and fluorescein. Studies have shown xanthones contain antimalarial and antibacterial properties, which makes them important compounds in pharmacology and other research.[11,12] Further research suggests xanthones contain chemopreventive and chemotherapeutic properties as well.[13]

As shown in Figure 2, the purpose of this research was to synthesize a dihydroxy-xanthone (2) from a tetrahydroxybenzophenone (1), which would then be methylated to produce the final product, 3,6-dimethoxyxanthone (3). This product can be used in future pharmaceutical research to determine its role in chemoprevention. In addition, (3) may be utilized as an intermediate to synthesize dyes such as fluorescein and rhodamine, important compounds in biochemical research.[14]

3,6-DIHYDROXYXANTHONE

The general reaction scheme (Figure 2) begins with (1), a common ultraviolet absorber found in sunscreens and cosmetics.[15,16] This compound is converted to (2) using microwave radiation with a sodium acetate catalyst (200 °C for 30-40 min at 30 bar, yield of >93% and purity of 99%, as determined by HPLC.)

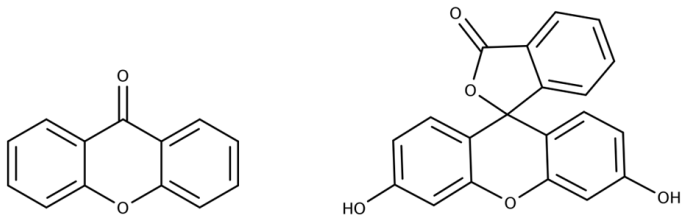
3,6-DIMETHOXYXANTHONE

The 3 and 6 positions of compound (2) were readily methylated using dimethyl sulfate (DMS), and the resulting methoxy groups were reported to be both relatively stable and easily removable.[17,18] The reactants and products were protected from light exposure because of possible photodegradation. The reaction was quenched by cooling to 0°C and addition of an excess of aqueous ammonia. The result was synthesizing (3) in over 92% yield and purity >99%. The structure and purity were determined by TLC, FTIR, ^{1}H NMR, HPLC, GCMS, and melting point.

Methods and Results

MATERIALS

Many of the raw materials used are associated with skin, eye, and respiratory system irritation; therefore, proper PPE was always utilized. The dimethyl sulfate (DMS) in step 2 has severe inhalation risks. As a precaution, this reagent was used under a fume hood and transferred using syringe technique between sealed, evacuated, and nitrogen-purged containers. According to the reagents' Certificate of Analyses, the purities of (1) and sodium acetate were 99.5% and 98%, respectively. Sodium acetate and (1) were evaluated by FTIR, HPLC, and melting point.[19,20,21,22]



xanthone *fluorescein*

Figure 1. General structure of a xanthone and fluorescein.

3,6-DIHYDROXYXANTHONE (2)

Preparation

A 25 mM sodium acetate solution was prepared. Then, 1 mmol of (1) (0.24621g) and 4 mL of the 25 mM sodium acetate solution (aqueous) were placed in a pre-weighed, empty microwave vessel (10-mL) equipped with a stir bar. The sealed vessel was irradiated in a CEM Discover SP microwave using a dynamic method which varies the power to maintain set parameters.[23] The general conditions for the reaction were: 200 °C, 30 bar, with low stirring for 20 min. When the reaction was complete, the vessel was removed from the microwave, vented, and allowed to cool. The contents of the vessel were then diluted in hot deionized water and stirred to break apart any clumps, which yielded a suspension. Then, the product, 3,6-dihyroxyxanthone (2), was filtered, using vacuum aspiration and a Buchner funnel with dried and weighed filter paper. The filter paper with (2) was placed in a weighed evaporating dish, covered with a watch glass, and dried *in vacuo* at 54 mmHg and 20 °C for 1 h.[24]

Modifications of the general reaction were made in the scale of the reaction, concentration of the base, pressure, and duration of irradiation. The temperature, wattage, and stirring speed remained constant between the different runs. The average yield for the 1 mmol scale was 79% and the 6 mmol scale 97% (Table 1).

Characterization

An analog melting point apparatus was used to determine the melting point of (2) in triplicate. The product began decomposing above 340 °C, slightly higher than the literature's 330 °C.[25,26] Compound (2) was compared to (1) using TLC (2:1 (v/v) solution of ethyl acetate and heptane).[27] Visualization under short and long wavelength UV light, as well as I_2 staining, showed no impurities (R_f (1) = 0.62, R_f (2) = 0.41).[28] The FTIR spectra for (2) were compared to literature FTIR results (Table 2).[26,29,30,31] To obtain the ^{1}H NMR spectrum, ≈50 mg of powdered (2) was dissolved in 2 g DMSO-d6.[32] Table 3 compares the

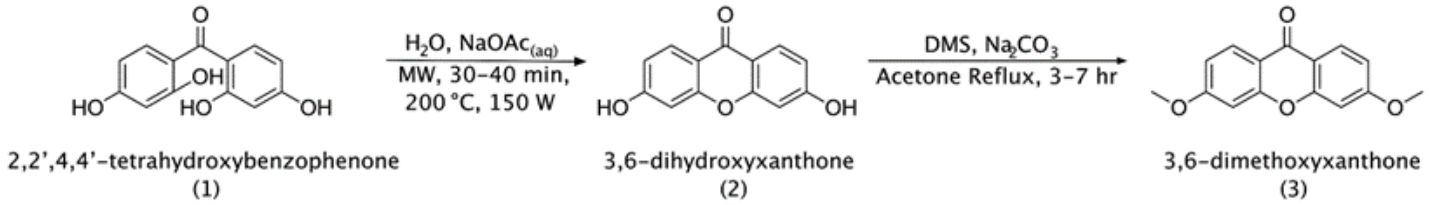


Figure 2. Overall reaction scheme

Table 1. List of the reaction conditions for each run of Step 1, including the amount of the starting material (1), the concentration and amount of NaOAc (base), time, pressure, and yield.

Run	SM (1), mmol	NaOAc conc., M	$NaOAc_{(aq)}$, mL	t, min	P, bar	% Yield
1	1	0.025	4	12	30	90
2	1	0.050	4	12	30	68
3	6	0.025	24	20	20	98
4	6	0.025	24	20	20	>99
5	6	0.025	24	40	20	97
6	6	0.050	24	40	20	95
7	6	1.219	24	40	20	97
8	6	1.219	24	40	20	96
9	6	0.100	24	30	20	97
10	6	0.100	24	30	20	97

Table 2. Frequencies in the FTIR spectrum of (2) compared to the literature values for (2).[26]

Lit., cm^{-1}	Expt., cm^{-1}	Lit., cm^{-1}	Expt., cm^{-1}
3382	3382	1254	1255
3095	3105	1243	1241
1629	1630	1169	1171
1611	1612	1115	1117
1575	1577	1104	1103
1454	1455	986	986
1352	1354	847	847
1324	1325	831	831
1291	1292	790	791
1273	1275	-	-

Table 3. Peaks in the ^{1}H NMR spectrum of (2) compared to the literature values.[26]

Lit, ppm	Integration	Expt., ppm	Integration
6.82	2H	6.84	2H
6.86	2H	6.95	2H
7.98	2H	8.03	2H
10.82	2H	10.82	2H

experimental and calculated results.[25,31] The purity of (2) was assessed by HPLC.[33] (70/30 methanol/aqueous acetic acid (1% v/v) @ 0.500 mL/ min, l= 273 nm.)[34,35] Two samples were prepared and run sequentially: 0.5 mg of (2) in 1 mL MeOH and 0.3 mg of (1) with 0.3 mg of (2) in 1 mL MeOH. Injected vol = 20 µL. $R_t(1)$ = 5.167 min, $R_t(2)$ = 9.943 min. The analysis indicated that (2) was over 99% pure. GCMS (2) was attempted using a solution containing 0.5 mg (2) in 1 mL MeOH.[36,37,38] However, since the melting point of (2) (330 °C) exceeds the maximum temperature of the column (300 °C), the results were inconclusive. No starting material (1) was evident, though.

3,6-DIMETHOXYXANTHONE (3)

Preparation

Acetone was distilled and dried using standard procedures, and a 0.5 M aq. ammonia solution was prepared.[39,40,41,42,43,44] In a sealed 50-mL volumetric flask, 6.3 mL of DMS was mixed with 23.7 mL of acetone.[45,46] The flask was then evacuated and purged with N_2.

In a 150-mL 3-neck round-bottom flask, anhydrous sodium carbonate (3.30 g, 23.9 mmol) and (2) (0.84 g, 3.68 mmol) were mixed in 75 mL acetone.[47,48] Reaction mixture was sealed, evacuated, and purged with N_2. The mixture was stirred for 30 min. (RT), 30 mL DMS/ acetone solution was added to the reaction mixture dropwise, by syringe over one hour then stirred for an additional hour at 20 °C and refluxed for 6 hours. The reaction was monitored by TLC every hour until the reaction was complete. While stirring, the reaction mixture was cooled to 0 °C and quenched by adding 15 mL of 0.5 M aqueous ammonia, dropwise, by syringe. The mixture was stirred for an additional hour at 20 °C. Due to the photosensitivity of the product (3), exposure of the reaction mixture to light was minimized using aluminum foil. After this stirring period, 100 mL of distilled water (rt) was added to the reaction mixture to form a white precipitate, which was then washed and filtered with 10 mL distilled water in triplicate. The filtered solid was dried under reduced pressure and weighed. Some product remained in the filtrate. To recover (3), the aqueous filtrate was concentrated down to remove the acetone, diluted with ethyl acetate, and transferred to a separatory funnel.[49] The organic layer was dried over magnesium sulfate, and evaporated under reduced pressure to yield (3) as a white solid.

Modifications of the general reaction were made involving changes to the scale and duration of the reaction. The amount of anhydrous sodium carbonate, DMS, and aqueous ammonia were adjusted relative to the amount of the starting material (2). The average percent yield was 94% (Table 4).

Characterization

The reaction was monitored hourly by TLC (2:1 (v/v) ethyl acetate to heptane).[27] Each plate had a lane for the starting material (2) and for the reaction mixture. (R_f (2) = 0.49, R_f(3) = 0.85). During the reaction, a third spot (possibly the monosubstituted intermediate) with an R_f of 0.66

Table 4. lists the conditions for each run and their percent yields for step 2.

Run	SM (2), mmol	Reflux time, hr.	% Yield
1	1.23	3	92
2	3.68	18	81
3	1.23	3	91
4	3.68	5	99
5	7.36	7	99
6	28.5	5	>99

was also present but disappeared by the end of the reaction. This was taken as an indication that the reaction was complete, as illustrated in Figure 3. The melting point of (3) ranged from 188-191 °C (*Lit* 187-188 °C).[50,26] Table 5 compares the peaks found in the experimental and literature spectra.[26] To obtain the ^{1}H NMR spectrum of (3), 50 mg (3) was dissolved in 2 g CD_2Cl_2. Table 6 compares the peaks found in the experimental and literature spectra.[26] The purity of (3) was assessed by HPLC (70:30 methanol: aqueous acetic acid (1% v/v); 0.500 mL/min; l= 273 nm; 0.50 mg/mL (3) in MeOH; R_t (1) = 5.300 min. (not observed), R_t (2) = 9.943 min. (not observed), R_t (3) = 26.100 min; results (3) was >99%)[33] and GCMS (0.25 mg (3) in 1.5 mL MeOH; 4.0 min @ 40 °C, 10 min @ 300 °C (ramp 40 °C/min); 256.0 m/z; R_t (3) =11.787 min).

Discussion

3,6-DIHYDROXYXANTHONE

3,6-dihydroxyxanthone (2), was synthesized via microwave-assisted sodium acetate-catalyzed annulation. This was verified using TLC, which showed two distinct R_f values for 2,2'4,4'-tetrahydroxybenzophenone (1) and (2): 0.62 and 0.41, respectively, as shown in Figure 3 (2) has a lower R_f value, reflecting its greater polarity. A potential explanation could be (2) is less soluble due to its restricted structure. Conversely, (1) has a flexible structure, which generally increases solubility.[51]

The FTIR and ^{1}H NMR spectrum of (2) showed a high similarity to the literature peaks (Tables 2 and 3). The calculated FTIR spectrum accurately predicted many of the peaks; however, strong, broad peaks that were present in the literature and experimental spectra were absent in the calculated prediction. The predicted ^{1}H NMR of (2) omitted the highest peak found in the literature and experimental spectrum. The HPLC showed a distinct peak at 9.94 min. with a shoulder at 9.19 min. The shoulder was present in every HPLC, in the same proportion, yielding the conclusion that they are from the same substance. The integration of this peak revealed (2) was over 99% pure.

It was determined that (2) began decomposing at 340 °C, which was higher than that of the literature (330 °C).[26] The high melting point also affected the GCMS results in that the product was not observed. It did verify, however, that the starting material was not present. The average yield for (2) was 93%, and yield was maximized to 99% by increasing the scale of the reaction to 6 mmol as summarized in Table 1.

While literature microwave parameters were ineffective, increases in time and temperature yielded (2) (Ref.: MW, 8-12 min, 90-130 °C; Actual: >20 min, 200 °C).[52] Catalyst concentrations from 0.025-1.2 M NaOAc provided good yield.

3,6-DIMETHOXYXANTHONE

Using the method describe above, (3) was synthesized via reflux (3-7 h). As the reaction progressed, TLC indicated consumption of (2) corresponded to appearance of (3) and an additional compound assumed to be the monosubstituted intermediate. After 3 - 4 hours, the monosubstituted compound was absent, indicating the reaction was complete, and (3) was less polar than (2) as predicted (R_f (2) = 0.49, R_f (3) = 0.85, Figure 3). The experimental FTIR and ^{1}H NMR spectra were compared to the literature, which revealed similarity between the peaks (Tables 5-6). The average yield for (3) was 92%. However, the greatest yield was 99%, which was obtained by increasing the ratio of starting materials and closely monitoring the temperature to ensure consistency. The HPLC revealed the purity of the product to be >99%. The bulk of

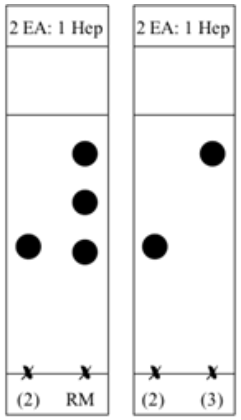


Figure 3. Depicts TLC plates run in 2:1 Ethyl Aetate:Heptane solution, showing (2) and the reaction mixture (RM) (3). The first plate is mid-reaction showing the monosubstituted intermediate, and the second plate depicts the end results.

Table 5. Frequencies in the FTIR spectrum of the experimental (3) compared to the literature values.

Lit, cm^{-1}	Expt., cm^{-1}	Lit, cm^{-1}	Expt., cm^{-1}
1612	1612	1099	1100
1501	1495	1018	1019
1428	1432	979	981
1357	1358	925	926
1302	1308	825	832
1257	1257	763	765
1211	1212	663	665
1157	1156	-	-

Table 6. Peaks in the ^{1}H NMR spectrum of (3) compared to the literature values.

Lit., ppm	Expt., ppm	Integration
3.90	3.83	6H
6.84	6.77	2H
6.90	6.91	2H
8.13	8.07	2H

(3) was donated to be used in further screening and research, i.e. to determine its anti-tumor properties, as similar xanthones have shown promising results.[53] Other research indicates xanthones similar to (3) have cytotoxic and anti-inflammatory effects; therefore, it may act as an antibacterial agent.[54] In addition, (3) can be used as an intermediate for fluorescent dyes, an important tool used to detect biological molecules.[55]

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