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Laboratory note

An improved synthesis of 5,6-dimethylxanthenone-4-acetic acid (DMXAA)

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Abstract

5,6-Dimethylxanthenone-4-acetic acid (DMXAA) is a novel anticancer agent with a number of unique activities, and is in clinical trial. The current synthesis of DMXAA involves six steps, beginning with a heterogeneous reaction to form an isonitrosoacetanilide, and gives an overall yield of 11% from 2,3-dimethylaniline. We report an alternative synthesis of the key intermediate 3,4-dimethylanthranilic acid via nitration of 3,4-dimethylbenzoic acid and separation of the key desired isomer by ready crystallisation. This, together with improvements in the rest of the synthesis, provide a shorter and higher-yielding route to DMXAA (22% overall from 3,4-dimethylbenzoic acid).

Keywords: DMXAA; Synthesis; Anti-vascular agent; Selective nitration

1. Introduction

5,6-Dimethylxanthenone-4-acetic acid (DMXAA, **8**) [1] is a novel anticancer agent currently in clinical trial, with a number of unique activities, including the ability to shut down blood flow in tumors [2,3], induce production of tumor necrosis factor [4,5], and inhibit tumor angiogenesis [6]. It shows curative antitumor activity in colon 38 tumors in mice, activity that is potentiated by a number of other agents, including radiation [7], the serotonin receptor antagonist cyproheptadine [8], immunotherapy [9], and enzyme–prodrug therapy [10].

The reported synthesis of DMXAA (Fig. 1) [1] involves the key intermediate 3,4-dimethylbenzoic acid (**4**). This is prepared from 2,3-dimethylaniline (**1**) by condensation with hydroxylamine and chloral hydrate to give the isonitrosoacetanilide (**2**), followed by acid-catalysed ring closure to the isatin (**3**) and oxidative ring opening of this to give **4** in 37% overall yield for the three steps. However, this method requires use of the toxic reagents hydroxylamine and chloral hydrate. The first step of this synthesis is a heterogeneous reaction, requiring a careful time-temperature profile, and producing a crude product (**2**) of low purity that is difficult to clean up. In this paper we report an alternative synthesis of **4** from a readily-accessible precursor, together with improvements in the subsequent synthesis of DMXAA.

2. Results and discussion

The best alternative route to **4** appeared to be via direct nitration of 3,4-dimethylbenzoic acid (**9**), followed by reduction. The nitration of 3,4-dimethylbenzoic acid (**9**) with KNO_3–H_2SO_4 fuming nitric acid was reported [11] to give a mixture of the three nitro isomers (**10a**–**10c**) in a ratio of 1:2:1 (Fig. 2). We repeated this work using fuming HNO_3, and obtained the same mixture of isomers in 94% overall yield, with a more desirable product ratio, determined by ^{1}H-NMR as 1:8:2. Crystallisation of this mixture by neutralisation of a hot aqueous solution of the sodium salts with acetic acid removed **10a**, giving a mixture of **10b** and **10c** only, in the ratio 3.4:1. Hydrogenation of this mixture, followed by crystallisation of the resulting mixture of amino

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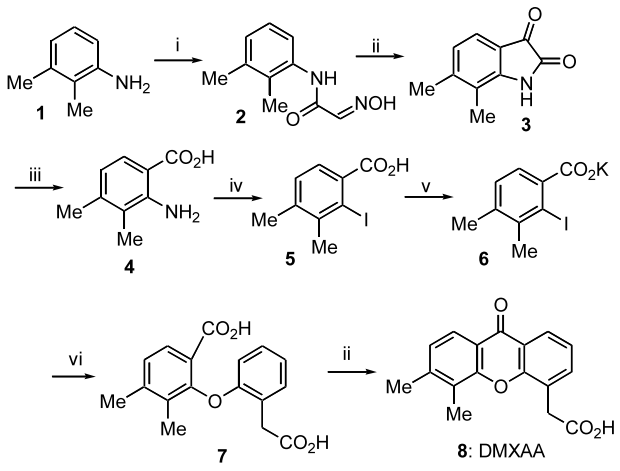
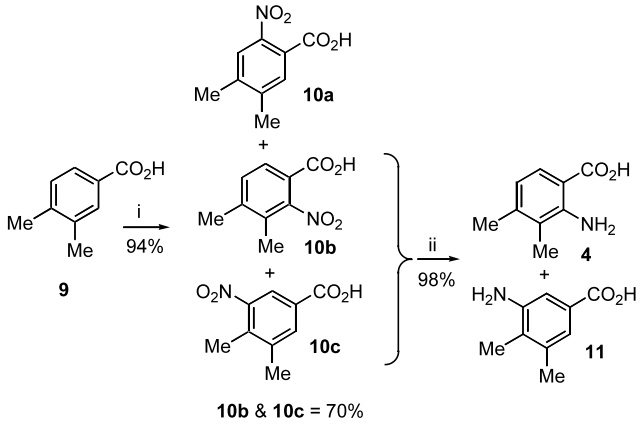


(i) $Cl_3CCH(OH)_2/NH_2OH$; (ii) H_2SO_4; (iii) H_2O_2/KOH; (iv) HNO_2/KI; (v) KOH; (vi) 2-hydroxyphenylacetic acid/Cu/TDA-1

Fig. 1. Original synthesis of DMXAA



(i) f.HNO_3; (ii) H_2/Pd/C.

Fig. 2. Alternative route to anthranilic acid **4**

compounds **4** and **11** by neutralisation of a hot aqueous solution of the sodium salts with acetic acid gave **4** (96% pure, 42% overall yield from **9**).

Together with this alternative synthesis of **4**, we also report on improvements to the reported [1] synthesis of DMXAA from **4**. These experiments are on a larger scale, using **4** prepared as both above and also as previously [1]. Diazotisation of **4** as previously [1], but using sulphamic acid to remove excess $NaNO_2$ before treatment with KI, improved the yield of 3,4-dimethyl-2-iodobenzoic acid (**5**) from 69 to 78%. This gives an overall yield of **5** from starting material (over three steps) of 33%, compared to 25% (over four steps) by the isonitrosoacetanilide route. Coupling of **5** obtained by this method with 2-hydroxyphenylacetic acid to give **8** was improved by rigorous drying of the DMSO and the tris[2-(2-methoxyethoxy)ethyl]amine catalyst over microwave-heated molecular sieves, raising the yield from 63 to 71%. Traces of the isomeric *meta*-iodoacid (from **11**) in the preparation of **5** did not couple, ensuring isomerically pure **7**. Finally, careful control of the water content to avoid sulphonated byproducts raised the yield of DMXAA from **7** in the sulphuric acid ring closure reaction from 70 to 93%. Overall, the yield of DMXAA by this new route from 3,4-dimethylbenzoic acid (**9**) was 22%, double that (11%) reported previously from 2,3-dimethylaniline (**1**) [1].

3. Experimental

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ. M.p.s were determined on a digital melting point apparatus, and are as read. NMR spectra were measured at 400 or 200 (^{1}H) and 100 MHz (^{13}C), and are referenced to Me_4Si. Mass spectra were recorded at nominal 5000 resolution. HPLC of mixed nitroacids (**10**) was conducted on a Bondclone C18 10 μm column of 3.9 × 300 mm, at a flow rate of 1 ml min^{-1}, using a gradient elution profile of A; 10–100%: B; 90–0% over 25 min. Solution A: 80% MeCN–H_2O. Solution B: 0.045 M ammonium formate, pH 3.5.

3.1. Nitration of 3,4-dimethylbenzoic acid (*9*)

3,4-Dimethylbenzoic acid (**9**) (5.0 g, 33 mmol) was added portionwise to stirred fuming HNO_3 ($d = 1.52$, 17 mL) maintained below −5 °C. The mixture was stirred at 0 °C for a further 10 min, then poured into water (250 mL). After cooling to 0 °C the precipitated solid was collected, washed with ice-cold water and dried to give a mixture of 4,5-dimethyl-2-nitrobenzoic acid (**10a**), 3,4-dimethyl-2-nitrobenzoic acid (**10b**) and 3,4-dimethyl-5-nitrobenzoic acid (**10c**) (6.11 g, 94%) in the approximate ratio of 1:8:2 as determined by ^{1}H-NMR. The mixture was dissolved in water (55 mL) containing NaOH (1.33 g) and the hot solution was treated with AcOH (3.0 mL) and then cooled slowly to 0 °C. After 2 h at 0 °C the precipitated solid was collected and washed with ice-cold water and dried to give a mixture of **10b** and **10c** (4.52 g, 70% yield based on **9**), in the approximate ratio of 3.4:1, as determined by ^{1}H-NMR. Compound **10b**: ^{1}H-NMR [$(CD_3)_2SO$]: δ 7.78 (d, J = 7.9 Hz, 1H,), 7.50 (d, $J = 8.0$ Hz, 1H), 2.38 (s, 3H), 2.12 (s, 3H). Compound **10c**: ^{1}H-NMR [$(CD_3)_2SO$]: δ 8.12 (d, $J = 1.2$ Hz, 1H), 8.03 (s, 1H), 2.41 (s, 3H), 2.36 (s, 3H).

3.2. Reduction of mixed nitroacids (*10*)

A solution of the mixed isomers **10b** and **10c** (4.52 g, 23.2 mmol) in MeOH (30 mL) was hydrogenated over 5% Pd–C at 55 psi for 10 h, and the catalyst and solvent

were removed to provide a mixture of 3,4-dimethylanthranilic acid (**4**) and 3-amino-4,5-dimethylbenzoic acid (**11**) as a solid (3.75 g, 98% yield). This mixture was dissolved in 1.0 N aq. NaOH (23 mL, 23 mmol) and water (27 mL), and the hot solution was clarified by filtration and then treated with AcOH (1.40 mL, 24 mmol). Following cooling at 0 °C for 2 h, the product was collected, washed thoroughly with ice-cold water and dried to give **4** (2.34 g, 42% overall yield from **9**) (96% pure by HPLC; see conditions above). ^{1}H-NMR [$(CD_3)_2SO$]: δ 7.51 (d, $J = 8.2$ Hz, 1H), 6.40 (d, $J = 8.2$ Hz, 1H), 2.21 (s, 3H), 2.00 (s, 3H).

3.3. 3,4-Dimethyl-2-iodobenzoic acid (*5*)

Finely powdered **4** (100 g, 0.605 mol) was added to a mixture of concd. H_2SO_4 (215 mL) and water (1270 mL). The mixture was stirred at 90 °C until homogeneous, then cooled to 0 °C. The stirred mixture was treated dropwise, below the surface, with a solution of $NaNO_2$ (46.8 g, 0.678 mol) in water (100 mL) at 0–2 °C over a 1 h period. The reaction mixture was stirred for a further 15 min at 0–2 °C, then treated with sulphamic acid (7 g) and stirred for 15 min. A cold solution of KI (340 g) in water (200 mL) was added in one portion to the cold (2 °C) diazotised solution. After the addition the stirred mixture was warmed up to room temperature (r.t.) over a period of 1 h, then gradually heated to 75 °C over a period of 2 h, and kept at 75 °C for a further 30 min. After addition of $Na_2S_2O_5$ (5 g), the reaction mixture was cooled to r.t. and the solid was collected, washed well with cold water, and dried to give a crude product (155 g) which contained (HPLC) 78% of **5** and 13% of 3,4-dimethyl-2-hydroxybenzoic acid. The crude product was used directly. Extraction of the mixture with hot diisopropyl ether gave pure **5**: m.p. 139–140 °C. ^{1}H-NMR [$(CD_3)_2SO$]: δ 13.14 (s, 1H, CO_2H), 7.22 (q, 2H, H-5,6), 2.43 (s, 3H, CH_3), 2.35 (s, 3H, CH_3). HPLC: 97%. Anal. Calc. for $C_9H_9IO_2$: C, 39.2; H, 3.3; I, 46.0. Found: C, 39.6; H, 3.3; I, 45.6%.

3.4. 2-[2-(Carboxylmethyl)phenoxy]-3,4-dimethylbenzoic acid (*7*)

The above crude acid **5** (100 g, 0.362 mol) was dissolved in a solution of KOH (23.4 g, 0.417 mol) in water (450 mL). The solution was concentrated under reduced pressure at 100 °C, and the resulting solid was heated under vacuum at 100 °C for 12 h to give the dry potassium salt (**6**). Sodium metal (13.6 g, 0.591 mol) was dissolved in MeOH (400 mL), then 2-hydroxyphenylacetic acid (45 g, 0.295 mol) was added, and the solution was evaporated to dryness under reduced pressure, then heated under vacuum at 100 °C for 24 h to give the anhydrous disodium salt. This was dissolved in anhydrous DMSO (350 mL, dried over microwave-dried molecular sieve) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1; 95%, 12 mL, dried over molecular sieves) was added. The mixture was stirred at 20 °C with the exclusion of moisture until homogeneous. Copper(I) chloride (7 g) and the powdered **6** (72 g, 0.229 mol) were then added, and the reaction mixture was stirred at ca. 40 °C until solidification occurred. The mixture was then heated in an oil bath at 85 °C for 4 h, until homogeneous. After cooling to r.t., the mixture was diluted with a solution of NaOH (4 g) in water (2.7 L), filtered through a Celite pad, and then acidified with glacial AcOH (200 mL). The precipitated solid was collected, washed well with water, and dried under vacuum at 100 °C to give a crude product (60 g, 87%). This was suspended in boiling MeOH (400 mL), hot EtOAc (400 mL) was added, and the resulting homogeneous solution was boiled down to 350 mL, during which time the product separated. After cooling at −5 °C overnight, the solid was collected, washed with cold EtOAc and dried to give 2-[2-(carboxylmethyl)phenoxy]-3,4-dimethylbenzoic acid (**7**) (49 g, 71%), which was used directly. A sample recrystallised from EtOAc had m.p. 240–242 °C. ^{1}H-NMR [$(CD_3)_2SO$]: δ 12.40 (s, 2H, CO_2H), 7.63 (d, $J = 7.9$ Hz, H-6), 7.27 (dd, $J = 7.4$, 1.2 Hz, H-6′), 7.20 (d, $J = 7.9$ Hz, 1H, H-5), 7.06 (m, 1H, H-4′), 6.90 (dd, $J = 7.40$, 7.40 Hz, 1H, H-5′), 6.09 (d, $J = 8.13$ Hz, 1H, H-3′), 3.70 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 2.00 (s, 3H, CH_3). HPLC: 99.5%. Anal. Calc. for $C_{17}H_{16}O_5$: C, 68.0; H, 5.4. Found: C, 68.1; H, 5.3%.

3.5. 5,6-Dimethyl-9-oxo-9H-xanthen-4-yl)acetic acid (DMXAA) (*8*)

Powdered crude **7** (100 g, 0.333 mol) was added to a stirred mixture of concd. H_2SO_4 (1300 mL) and water (478 mL). The mixture was heated to 90 °C over a 30 min period, and maintained this temperature until homogeneous and for a further 25 min. The mixture was then cooled to 10 °C, stirred well, and diluted slowly with water (6 L). The precipitated solid was collected, washed with water (8 L) and suspended in a mixture of MeOH (1.5 L) and water (1 L), with the exclusion of light. Addition of concd. aq. NH_3 (ca. 20 mL) gave a homogeneous solution, that was immediately acidified with glacial AcOH (1 L), the precipitated product was collected, washed with water (10 L), Milli-Q water (6 L), cold MeOH (500 mL), and dried under vacuum at 100 °C to give **8** (87 g, 93%): m.p. 259–260 °C. ^{1}H-NMR [$(CD_3)_2SO$]: δ 12.63 (brs, 1H, CO_2H), 8.09 (dd, $J = 7.9$, 1.6 Hz, 1H, H-1), 7.90 (d, $J = 8.1$ Hz, 1H, H-8), 7.78 (dd, $J = 7.2$, 1.5 Hz, 1H, H-3), 7.40 (dd, $J = 7.6$, 7.6 Hz, 1H, H-2), 7.27 (d, $J = 8.1$ Hz, 1H, H-7), 3.97 (s, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.35 (s, 3H, CH_3). HPLC: 98.0%. Anal. Calc. for $C_{17}H_{14}O_4$: C, 72.3; H, 5.0. Found: C, 72.5; H, 5.3%.

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