SYNTHESIS OF (*Z*)-3-BENZYLIDENE-3,4-DIHYDRO-2-OXO-2*H*-1,4-BENZOXAZINE-5-CARBOXYLIC ACID, A NATURALLY OCCURING INHIBITOR OF GLUTATHIONE *S*-TRANSFERASE.

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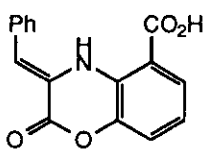
Abstract - Preparation of a number of 2*H*-1,4-benzoxazin-2-ones has been effected by reaction of substituted 2-aminophenols with methyl 3-phenylpyruvate. This methodology has been applied to afford a rapid and efficient synthesis of the naturally occuring glutathione *S*-transferase inhibitor (*Z*)-3-benzylidene-3,4-dihydro-2-oxo-2*H*-1,4-benzoxazine-5-carboxylic acid.

In 1992, Komagata *et al.*[1] isolated benzoxazinone (**1**) from a fermentation broth of *Streptomyces* sp. TA-3037 and found it to be an inhibitor of glutathione *S*-transferase, an enzyme implicated in drug resistance to cancer chemotherapy. The structure of (**1**) was assigned on the basis of ^{1}H and ^{13}C nmr studies and derivatisation to the methyl ester (**2**). Herein, we wish to report the total synthesis of benzoxazinone (**1**) and its methyl ester analog (**2**), confirming the original structural assignments to be correct and demonstrating a facile method to access this class of functionalised benzoxazinones.



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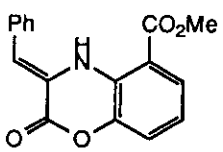
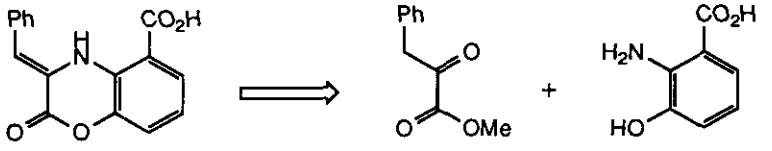


2

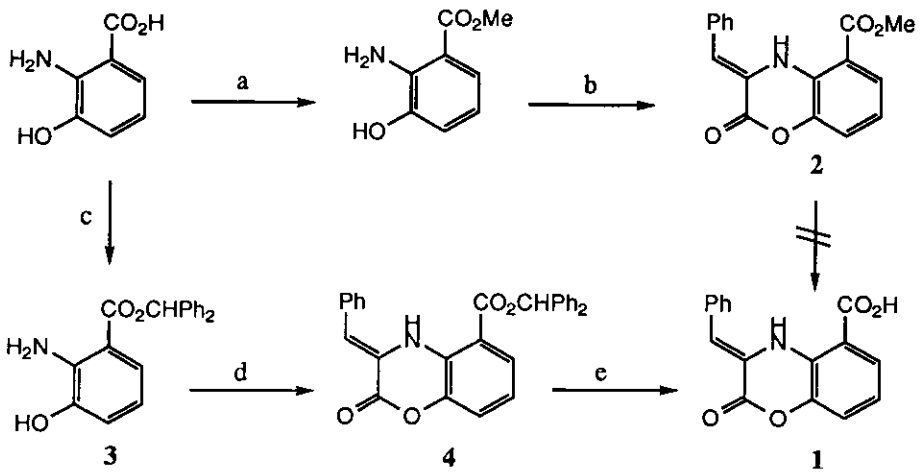
Our initial attempts to prepare benzoxazinone (**1**) examined the cyclocondensation of 2-aminophenols with α-keto esters, an established[2] method for the synthesis of the 2*H*-1,4-benzoxazin-2-one ring. The shortest route to the target involves reaction of commercially available 3-hydroxyanthranilic acid with methyl 3-phenylpyruvate[3] (Scheme 1). The acid, however, proved to be unreactive under a number of reaction conditions (condensation was attempted in toluene at reflux, water at reflux, and dimethylformamide at 100 °C) and was recovered from the reaction mixture, although the pyruvate ester had been consumed.



1

Scheme 1

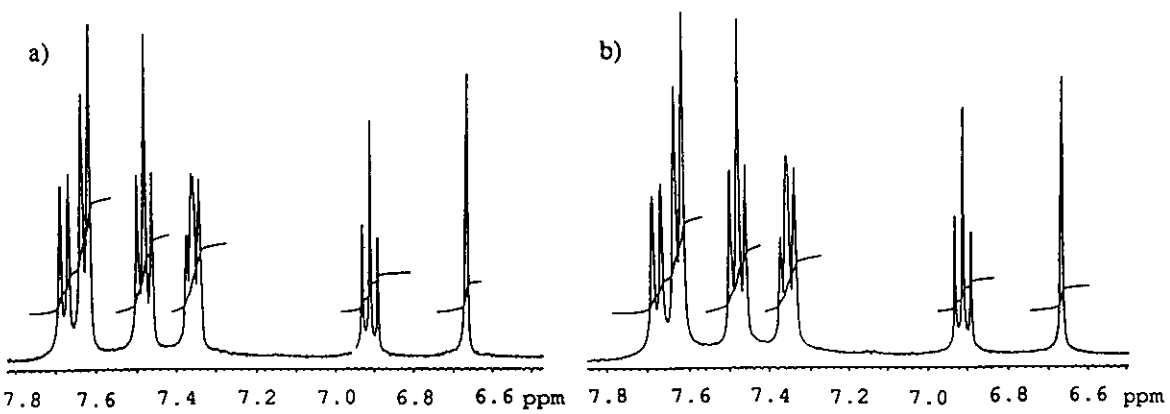
Hydrolysis of methyl ester (**2**) would generate the acid; thus, we investigated the preparation of this ester by cyclocondensation, hoping to achieve synthesis of the acid in a subsequent hydrolysis step. We were pleased to observe the reaction between methyl 2-amino-3-hydroxybenzoate[4] and methyl 3-phenylpyruvate afforded the desired benzoxazine (**2**) in 78% yield (Scheme 2), the product crystallising out of the reaction mixture on cooling. Unfortunately, however, we were unable to hydrolyze the methyl ester group: under basic conditions the benzoxazinone ring proved the more susceptible towards hydrolysis, and Lewis acids caused decomposition to yield unidentified degradation products. Thus we were forced to examine the use of acid-labile protecting groups to mask the carboxyl group of 3-hydroxyanthranilic acid. Benzhydryl esters have been reported[5] to be useful protecting groups for acids when compounds incorporate sensitive functionality.



a) MeOH, sat. HCl, Δ, 83%; b) $PhCH_2COCO_2Me$ (2 equiv), toluene, *p*-TsOH (0.05 equiv), 110 °C, N_2, 24 h, 78%; c) Ph_2CN_2 (1.1 equiv), DMF, room temperature, 48 h, 65%; d) $PhCH_2COCO_2Me$ (2 equiv), *p*-TsOH (0.05 equiv), 110 °C, N_2, 24 h, 53%; e) CF_3CO_2H, room temperature, 20 min, 64%.

Scheme 2

Esterification of 3-hydroxyanthranilic acid with diphenyldiazomethane[6] gave the benzhydryl ester (**3**) in 65% yield after chromatography; subsequent cyclocondensation with methyl 3-phenylpyruvate was then found to deliver the desired benzoxazinone (**4**) in 53% yield; the lower yield observed here is attributable to the greater steric influence of the benzhydryl group. Finally, removal of the benzhydryl group in trifluoroacetic acid afforded in 64% yield the desired benzoxazinone (**1**) which displayed physical and spectroscopic properties identical to those reported[1b] for this compound.



a) authentic benzoxazine (**1**), 400 MHz, DMSO-d_6; b) synthetic benzoxazine (**1**), 400 MHz, DMSO-d_6

In conclusion, we have demonstrated an efficient preparation of benzoxazinone (**1**) in only 3 steps from readily available starting materials, and confirmed the structural assignment by total synthesis.

EXPERIMENTAL

General. ^{1}H (400 MHz) and ^{13}C (100 MHz) nmr spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Gemini spectrometer. Ir spectra were recorded as KBr discs on a Nicolet 210 FT-IR instrument and uv spectra were obtained with a Varian-Cary 1E UV-Visible spectrophotometer. A Meltemp or Fisher-Johns apparatus was used for melting point determinations and they are uncorrected; elemental analyses were performed by Robertson Microlit Labs, Inc. Madison, New Jersey. Analytical tlc was performed on Whatman PE SIL G/UV polyester backed silica gel plates, and flash column chromatography employed silica gel purchased from Baxter Scientific Products (S/P brand silica gel 60Å, 230-400 mesh). All solvents and reagents were used as purchased.

Methyl (Z)-3-Benzylidene-3,4-dihydro-2-oxo-2*H*-1,4-benzoxazine-5-carboxylate (**2**).

To a stirred solution of methyl 2-amino-3-hydroxybenzoate[4] (0.250 g, 1.50 mmol) in toluene (30 ml) was introduced a solution of methyl 3-phenylpyruvate (2.59 ml of a 0.584 M solution in benzene, 1.51 mmol) and *para*-toluenesulfonic acid monohydrate (0.015 g, 0.075 mmol, 0.05 equiv). After 24 h at reflux, a second equivalent of methyl 3-phenylpyruvate was added and refluxing continued for a further 20 h. On cooling to room temperature, the benzoxazine (**2**) was observed to crystallise out of the reaction solution as fine yellow-green needles which were collected by filtration. A final recrystallisation from toluene afforded the pure product as yellow needles (0.345 g, 78%). mp 194.5-195.5 °C; R_f 0.81 (SiO_2/CH_2Cl_2); λ_{max} (EtOH) nm (log ε) 200 (4.46), 242 (4.38), 281 (4.01), 380 (4.23); ir υ_{max} (KBr) 3313, 1741, 1696 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 10.65 (1H, br s), 7.73 (1H, dd, *J* = 8.1, 1.5 Hz), 7.59 (2H, m), 7.51 (2H, m), 7.34 (1H, dt, *J* = 7.8, 1.2 Hz), 7.21 (1H, dd, *J* = 7.8, 1.5 Hz), 6.86 (1H, s), 6.82 (1H, t, *J* = 8.1 Hz), 3.93 (3H, s); ^{13}C nmr ($CDCl_3$) δ 167.5, 158.0, 140.4, 136.1, 130.2, 129.1, 128.2, 128.1, 126.9, 122.6, 120.9, 118.6, 112.0, 111.2, 52.3. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 68.95; H, 4.23; N, 4.49.

Diphenylmethyl 2-Amino-3-hydroxybenzoate (**3**).

To a stirred solution of 2-amino-3-hydroxybenzoic acid (0.150 g, 0.979 mmol) *in anhydrous* dimethylformamide (5.0 ml) at room temperature was introduced a solution of diphenyldiazomethane[6] (1.2 ml of a 0.92 M solution in tetrahydrofuran, 1.1 mmol). After 24 h stirring, the solvent was evaporated *in vacuo* and the residue subjected to chromatography (silica gel, neat dichloromethane eluant) to afford the product as an off-white solid (0.205 g, 65%). Recrystallisation from cyclohexane/benzene (4:1) gave a colorless crystalline solid suitable for elemental analysis. mp 129-130 °C; R_f 0.17 (SiO_2/CH_2Cl_2); λ_{max} (EtOH) nm (log ε) 202 (1.58), 223 (4.46), 252 (3.84), 346 (3.69); ir υ_{max} (KBr) 3509, 3377, 1655 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.70 (1H, dd, J = 8.0, 1.2 Hz), 7.44 (4H, m), 7.38-7.28 (6H, m), 7.08 (1H, s), 6.79 (1H, br d, J = 8.0 Hz), 6.52 (1H, t, J = 8.0 Hz), 5.90 (2H, br s), 4.68 (1H, br s); ^{13}C nmr ($CDCl_3$) δ 167.3, 143.5, 140.5, 140.2, 128.5, 127.8, 126.9, 122.9, 118.3, 115.7, 111.4, 77.1. Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; H, 5.36; N, 4.38. Found: C, 75.28; H, 5.49; N, 4.31.

Diphenylmethyl (*Z*)-3-Benzylidene-3,4-dihydro-2-oxo-2*H*-1,4-benzoxazine-5-carboxylate (**4**).

Into a dry flask were introduced methyl 3-phenylpyruvate (0.277 g, 1.55 mmol), diphenylmethyl ester (**3**) (0.500 g, 1.55 mmol) and *para*-toluenesulfonic acid monohydrate (0.010 g, 0.05 mmol, 0.03 equiv). Following addition of toluene (50 ml), the stirred solution was heated at 110 °C for 4 h, whereupon an additional equivalent of methyl 3-phenylpyruvate dissolved in 10 ml of toluene was introduced and the heating continued for a further 20 h. Evaporation of the solvent *in vacuo* gave a yellow solid which was subjected to chromatography (silica gel, neat dichloromethane) to afford an amorphous yellow solid. Final purification by crystallisation from cyclohexane/toluene (2:1) yielded the ester as bright yellow crystals (0.369 g, 53%). mp 178-179 °C; R_f 0.69 (SiO_2/CH_2Cl_2); λ_{max} (EtOH) nm (log ε) 201 (4.70), 243 (4.36), 284 (3.92), 350 (3.94), 386 (4.14); ir υ_{max} (KBr) 3338, 1746, 1685 cm^{-1}; ^1H nmr ($CDCl_3$) δ 10.70 (1H, br s), 7.93 (1H, dd, J = 8.0, 1.2 Hz), 7.57-7.30 (15H, m), 7.23 (1H, dd, J = 8.0, 1.2 Hz), 7.16 (1H, s), 6.87 (1H, t, J = 8.0 Hz), 6.85 (1H, s); ^{13}C nmr ($CDCl_3$) δ 166.1, 157.8, 140.4, 139.7, 133.9, 130.6, 129.1, 128.5, 128.2, 128.1, 128.0, 127.1, 126.7, 122.4, 121.0, 118.6, 112.1, 111.2, 77.4. Anal. Calcd for $C_{29}H_{21}NO_4$: C, 77.84; H, 4.73; N, 3.13. Found: C, 78.06; H, 4.66; N, 3.06.

(*Z*)-3-Benzylidene-3,4-dihydro-2-oxo-2*H*-1,4-benzoxazine-5-carboxylic acid (**1**).

To a flask containing benzoxazinone (**4**) (0.012 g, 0.03 mmol) was introduced trifluoroacetic acid (1.0 ml), and the resulting solution was stirred at room temperature for 20 min. Evaporation of the trifluoroacetic acid *in vacuo* afforded an orange-yellow solid which was heated to reflux in chloroform (2.0 ml) and collected by filtration after cooling to room temperature. Recrystallisation from methanol/chloroform (1:1) yielded the product as fine, yellow needles (0.005 g, 64%). mp (synthetic) 244-245 °C, mp (authentic[1]) 245-246 °C, mp (mixed) 244-245 °C; R_f 0.74 (SiO_2/EtOH), 0.76 (SiO_2/4:1:2 n-BuOH:MeOH:H_2O); λ_{max} (EtOH) nm (log ε) 202 (4.47), 241 (4.35), 281 (4.04), 381 (4.26); ir υ_{max} (KBr) 3421, 3333, 1747, 1514, 1439 cm^{-1}; ^1H nmr (DMSO-d_6) δ 13.81 (1H, br s), 11.00 (1H, br s), 7.68 (1H, d, J = 7.8 Hz), 7.63 (2H, d, J = 7.8 Hz), 7.48 (2H, t, J = 7.8 Hz), 7.36 (2H, m), 6.91 (1H, t, J = 7.8 Hz), 6.67 (1H, s); ^{13}C nmr (DMSO-d_6) δ 169.4, 157.3, 140.4, 134.1, 129.9, 129.1, 128.0, 127.9, 126.8, 123.3, 120.6, 118.8, 112.0, 109.5.

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