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Latent Inhibitors Part 11. The Synthesis of 5-Spirocyclopropyl Dihydroorotic Acid.

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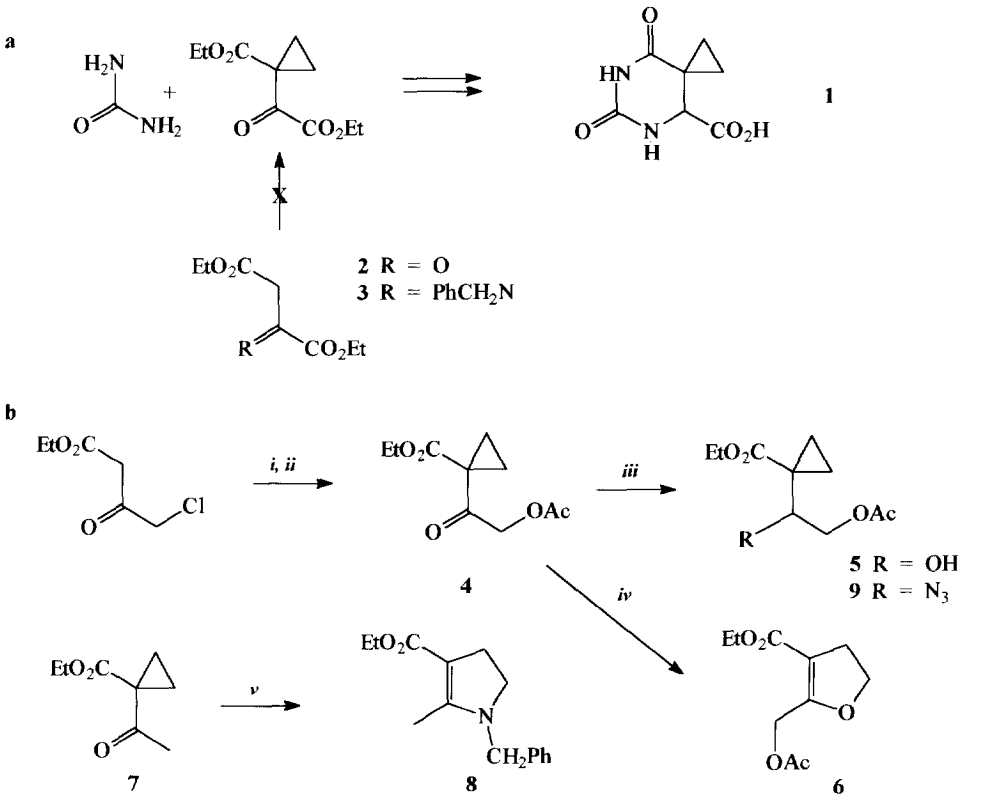
Abstract: Approaches to the synthesis of 5-spirocyclopropyldihydroorotic acid are described. Difficulties were encountered in the lack of reactivity of diethyl 2-oxobutandicarboxylate and its derivatives towards cyclopropanation. Potential intermediates were prepared from ethyl 4-acetoxy-3-oxobutanoate but rearrangements involving ring expansion of the cyclopropane ring and hindrance towards condensation reactions of the ketone prevented formation of the pyrimidine. A successful route involving Strecker synthesis of a precursor aminoacid from ethyl 1-formyl-cyclopropane-1-carboxylate in which the formyl group was converted into the corresponding the urea led to the target compound.

The inhibition of dihydroorotate dehydrogenase (DHOD) has been recognised as a significant target for chemotherapy especially of parasitic diseases[1,2]. We have shown that 5-spirocyclopropanobarbituric acid and some aryl and alkyl derivatives substituted in the cyclopropane ring are inhibitors of DHOD from *Clostridium oroticum*. Although these compounds showed interesting acitivity, the most direct substrate analogue that would contain the cyclopropane group for activation and irreversible inhibition is 5-spirocyclopropyldihydroorotic acid **1** itself. We have investigated the synthesis of this compound.

Following the normal procedures used for the preparation of spirocyclopropyl pyrimidines[2], the most direct approach to the synthesis of **1** in racemic form is by condensation of the appropriate cyclopropyl ketoester with a diamino precursor such as urea or thiourea (Scheme 1a). Accordingly, attempts were made to dialkylate diethyl 2-oxobutandioate **2** with 1,2-dibromoethane under many conditions (in aprotic solvents with strong bases or using phase transfer conditions) but without success. Similar alkylation of the benzylimine (**3**) of **2** was attempted but was not successful. In comparison with compounds that lack the electron withdrawing substituent adjacent to the ketone (e.g. ethyl acetoacetate) this lack of reactivity is surprising. We therefore considered that a latent carboxylic acid might be introduced into the synthesis and ethyl 1-(2-acetoxy-1-oxoethyl)cyclopropane-1-carboxylate **4** was prepared in two steps from commercially available ethyl 4-chloroacetoacetate (Scheme 1b).

Elaboration of **4** was attempted in several ways. Direct condensation with guanidine or thiourea was not successful. Reductive amination in the presence of ammonium acetate and sodium cyanoborohydride afforded notthe expected amino ester but the corresponding alcohol **5**. In order to develop a potential synthesis of the two



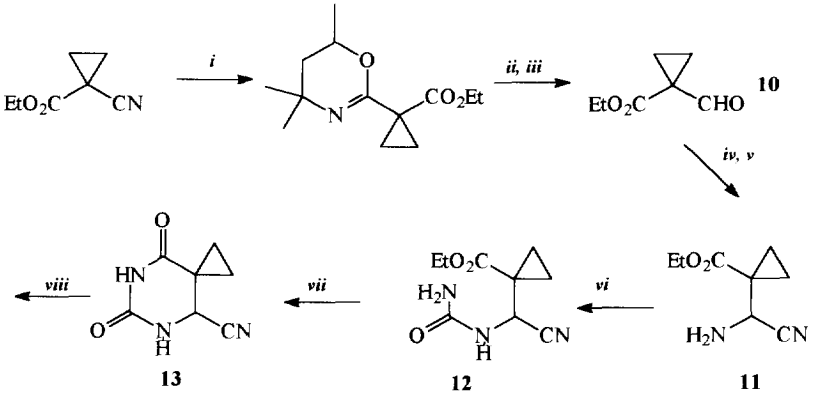
Scheme 1. Reagents: *i* NaOAc; *ii* $(CH_2Br)_2$, K_2CO_3, acetone; *iii* $NaBH_3CN$, NH_4OAc aq.; *iv* α-methylbenzylamine, $TiCl_4$; *v* $PhCH_2NH_2$

enantiomers of the target compound, the condensation of **4** with α-methylbenzylamine was attempted using titanium tetrachloride as catalyst, a reagent that is known to promote reactions at hindered ketones[3]. However, the only compound isolated and characterised from such reactions was the furan **6** in which the cyclopropane had undergone acid catalysed ring expansion.

Bearing in mind the fact that malonate derivatives condense satisfactorily with urea[2], the lack of reactivity of **4** was unexpected. Before embarking on further routes, we wished to establish the factors contributing to the unreactivity. Condensation of the intermediate ethyl 4-acetoxyacetoacetate, which lacks the cyclopropane ring, with benzylamine was unsuccessful. The problem became clearly associated with electron withdrawing subsitutents at C-4 with the observation that ethyl 1-oxoethylcyclopropane-1-carboxylate **7** condenses with benzylamine but spontaneously undergoes ring expansion to the N-benzylpyrroline **8**. Bearing in mind the unreactivity of the imine **3** it was not surprising that the benzylimine of ethyl acetoacetate did not undergo dialkylation with dibromoethane, a result consistent also with literature precedent[4].

One potentially useful intermediate has so far been obtained, the alcohol **5** and attempts were made to

convert the hydroxyl group into an amino group. Some encouraging results were obtained but although the azide **9** was obtained via the iodide prepared with triphenylphosphite methiodide[5] followed by sodium azide, the material was not sufficiently pure to use for cyclisation. None of the functional group modifications attempted on this alcohol went sufficiently cleanly. With all of these experimental problems, an alternative strategy was chosen using the Strecker synthesis. Ethyl 1-formylcyclopropane-1-carboxylate **10** was prepared by adaptation of a known route[6] (Scheme 2) and submitted to Strecker conditions.



Scheme 2. Reagents: H_2SO_4, 2-methylpentan-1,2-diol; *ii* $NaBH_4$; *iii* $(CO_2H)_2$; *iv* TMSCN, KCN, 18-crown-6; *v* NH_3 MeOH; *vi* NaOCN, HCl aq.; *vii* LDA, THF; *viii* HCl aq. 3M.

Traditional reaction conditions using sodium cyanide and ammonium acetate[7] were not successful but the use of potassium cyanide in the presence of 18-crown-6 together with trimethylsilylcyanide to trap the intermediate cyanohydrin followed by treatment with ammonia led to the aminonitrile **11**[8]. The amino group was converted into the urea **12** with sodium isocycanate in the presence of acid. Cyclisation under conditions shown to be successful with urea in our previous work[2] (sodium ethoxide in ethanol) or potassium t-butoxide in DMSO were unsuccessful but the cyanopyrimidine **13** was obtained using LDA in THF at -20°C. Finally, hydrolysis of the nitrile to the required carboxylic acid was carried out with aqueous hydrochloric acid. The biological properties of **13** and **1** will be reported elsewhere.

EXPERIMENTAL

Ethyl 4-acetoxy-3-oxobutanoate: A stirred mixture of ethyl 4-chloroacetoacetate (25 g, 0.15 mol), anhydrous sodium acetate (28.8 g, 0.46 mol) and dry acetic acid (160 ml) was heated at 85°C for three days. The acetic acid was distilled off and dichloromethane added (200 ml). The mixture was cooled to 0°C for 2 hours, the solid filtered off and the filtrate washed with water. The solution was then dried (Na_2SO_4) and the solvent evaporated. The pure product was obtained by distillation under reduced pressure (6.3 g; 22%), b.p. 102°C/1.4 mm (lit.[9] 88-89°C/2 mm) δ_H (90 MHz; $CDCl_3$), 1.30 (3H, t, $C\underline{H}_3CH_2$), 2.20 (3H, s, OCH_3), 3.53 (2H, s, $EtO_2CC\underline{H}_2$), 4.25 (2H, q, $CH_3C\underline{H}_2$), 4.82 (2H, s, CH_2OAc); ν_{max} (liq. film) 1730 (C=O), 1235 (C-O) cm^{-1}.

1-(2-Acetoxy-1-oxoethyl)-1-ethoxycarbonylcyclopropane: **4** Ethyl 4-acetoxy-3-oxobutanoate (2.0 g, 10.7 mmol), 1,2-dibromoethane (3.4 ml, 40 mmol), anhydrous potassium carbonate (6.3g, 46 mmol) and dry acetone (30 ml) were stirred at reflux for 20 hours. Ether (100 ml) was then added and the red mixture filtered through kieselguhr. The solvent was then removed and xylene added. This was then distilled off to ensure all the dibromoethane had been removed. Distillation gave the product (1.1 g, 49%) as a clear oil. b.p. 90°C/1 mm; δ_H (90 MHz; $CDCl_3$), 1.30 (3H, t, $C\underline{H}_3CH_2$), 1.64 (4H, s, cyclopropyl), 2.19 (3H, s, OCH_3), 4.25 (2H, q, $CH_3C\underline{H}_2$),5.22 (2H, s, CH_2OAc); ν_{max} (liq. film) 3020 and 2980 (C-H), 1725 (C=O), 1230 (C-O) cm^{-1}. Found m/z 214.214.830 (M^+), $C_{10}H_{14}O_5$ requires 214.0841.

1-(2-Acetoxy-1-hydroxyethyl)-1-ethoxycarbonylcyclopropane: **5** Ammonium acetate (22.7 g, 0.29 mol) was dissolved in acetic acid (65 ml) and then sodium cyanoborohydride (2.8 g, 0.04 mol) was added to the solution. The foregoing ester (6.34 g, 0.03 mol) was added dropwise and the solution stirred at room temperature for 2 hours. The acetic acid was then removed under reduced pressure and the remaining solution basified with sodium hydrogen carbonate. Extraction with ethyl acetate gave the product as a clear oil (6.49 g, 100%); δ_H (90 MHz; $CDCl_3$) 1.0-1.4 (7H, m, $C\underline{H}_3CH_2$ plus cyclopropyl), 2.12 (3H, s, $COCH_3$), 3.0 (1H, s, OH), 3.55 (1H, t, C<u>H</u>OH), 4.1-4.4 (4H, m, $C\underline{H}_2OAc$ plus $CH_3C\underline{H}_2$); ν_{max} (liq. film) 3500 (OH), 1710 (C=O) cm^{-1}; Found m/z 143.0722 (M^+-CH_2OAc, M^+ not observed) $C_7H_{11}O_3$ requires 143.0708.

Ethyl 1-cyano-1-cyclopropane carboxylate: Ethyl cyanoacetate (11.3 g, 0.1 mol), 1,2-dibromoethane (29 g, 0.2 mol) and potassium carbonate (40 g, 0.4 mol) were stirred together for 16 hours at 25°C. Water was then added and the organic compounds extracted with ether (3 x 100 ml). The ether layers were then combined, dried and evaporated under reduced pressure to leave an oil. Distillation gave the product (10.0 g, 72%), b.p. (60°C/0.9 mm lit[10]: 57°C/1.5 mm); δ_H ($CDCl_3$; 250 MHz) 1.35 (3H, t, $CH_2C\underline{H}_3$), 1.65 (4H, d, cyclopropyl), 4.25 (2H, q, $C\underline{H}_2CH_3$); ν_{max} (liq. film) 2990 (C-H), 2250 (C N), 1750 (C=O) cm^{-1}.

2(1[1-Ethoxycarbonylcyclopropane])-4,4,6-trimethyl-5,6-dihydro-1,3-oxazine: Concentrated sulfuric acid (8.5 ml) was cooled in an ice bath and the foregoing nitrile (5 g, 0.036 mol) was added over 45 minutes. 2-Methylpentan-2,4-diol (3.95 g, 0.067 mol) was then added dropwise over one hour, keeping the reaction cooled in the ice bath. The mixture was stirred for a further hour before pouring onto ice. The aqueous layer was washed with chloroform (3 x 50 ml) and then basified to pH 12 and 10M NaOH and the product extracted with ether. Drying (Na_2SO_4) and evaporation of the solvent gave a red oil. The crude product was purified by distillation (kugelrohr) to give the product (4.6 g, 57%), δ_H (250 MHz; $CDCl_3$), 1.07 (3H, s, CH_3-), 1.09 (3H, s, CH_3C-), 1.15 (3H, t, $CH_2C\underline{H}_3$), 1.25 (3H, d, $C\underline{H}_3CH$), 1.25-1.6 (6H, m, $C\underline{H}_2CH$ and cyclopropyl), 4.03 (2H, q, $C\underline{H}_2CH_3$), 4.10 (1H, m, $CH_3C\underline{H}$); ν_{max} (liq. film) 2970 (C-H), 1760 (C=O), 1670 (C=N) cm^{-1}.

1-(1-Amino-1-cyanomethyl)-1-ethoxycarbonyl cyclopropane: **11** To the foregoing dihydrooxazine (15 g, 0.067 mol) in THF (50 ml) and ethanol (50 ml) at -40°C was added alternately an alkaline solution of sodium borohydride (2.8 g, 0.123 mol) (in minimum volume of water, to which 1 drop of 10M NaOH had been added), and 10M HCl. The temperature was kept below -35°C and the pH between 6 and 8. The mixture was stirred at about -45°C for 1.5 hours before pouring into water (150 ml) and basifying with 10M NaOH. The solution was extracted with ether (3 x 50 ml), the organic extracts combined, dried (Na_2SO_4) and solvent evaporated to give the crude product as a clear

oil (15.9 g, 98%). The crude product was not purified further and was used directly in the next reaction. δ_H (90 MHz; $CDCl_3$), 1.17 (18 H, m, 3 x CH_3 plus cyclopropyl plus $C\underline{H}_2CH$ plus $C\underline{H}_3CH_2$), 2.65 (1H, d, NH), 3.6-4.3 (4H, m, NHC<u>H</u> plus OCH plus $C\underline{H}_2CH_3$).

Oxalic acid (7.4 g, 59 mmol) was dissolved in water (20 ml) and the foregoing tetrahydrooxazine (7.4 g, 31 mmol) was added. Steam distillation of this mixture was carried out until 300 ml of distillate had been collected. The distillate was saturated with sodium chloride and extracted with ether (4 x 50 ml). The organic extracts were dried (Na_2SO_4) and solvent removed under reduced pressure to give *ethyl 1-formylcyclopropane-1-carboxylate* **10** (2.6 g, 60%); δ_H (250 MHz:$CDCl_3$), 1.3 (3H, t, $C\underline{H}_3CH_2$), 1.55 (4H, d, cyclopropyl), 4.25 (2H, q, $CH_3C\underline{H}_2$), 10.39 (1H, s, CHO); ν_{max} (liq. film) 2290 (C-H), 1740 (C=O), 1710 (C=O) cm^{-1}.

The aldehyde **10** (1 g, 7.0 mmol), trimethylsilylcyanide (2.26 ml, 16.9 mmol), potassium cyanide (70.4 mg, 1.1 mmol), 18-crown-6- (70.4 mg, 0.3 mmol) and dichloromethane (14 ml) were stirred under nitrogen at room temperature for 18 hours. After this time, saturated methanolic ammonia solution (50 ml) was added and the solution again stirred for 24 hours at room temperature. Water was then carefully added and the solution stirred vigorously for 30 min. The organic layer was then collected and washed with water (4 x 50 ml), the organic layer separated, dried (Na_2SO_4) and the solvent removed. Silica chromatography (hexane:ethyl acetate, 70:30) gave the product as a clear oil (0.59 g, 50%) R_f 0.1, b.p. 100°C/0.4 mm; δ_H (250 MHz; $CDCl_3$), 1.26 (3H, t, $C\underline{H}_3CH_2$), 1.03 and 1.40 (4H, m, cyclopropyl), 2.03 (2H, s, NH_2), 3.57 (1H, s, CH), 4.18 (2H, q, $CH_3C\underline{H}_2$); ν_{max} (liq. film) 3400 and 3320 (primary NH_2), 2980 (C-H), 1750 (C=O) cm^{-1}; Found: C, 57.2; H, 7.3; N, 16.3. $C_8H_{12}N_2O_2$ requires C, 57.1; H, 7.1; N, 16.7%.

Hexahydro-2,6-dioxo-5-spirocyclopropyl-4-pyrimidine-6-carbonitrile: **13** The foregoing aminonitrile **11** (1.27 g, 7.56 mmol) and sodium isocyanate (0.70 g, 10.8 mmol) were stirred together in 1M HCl (8 ml) at room temperature for 1.5 hours. After this time the white solid product (*1-(1-cyano-1-ureidomethyl-1-ethoxycarbonyl cyclopropane* **12**) was filtered off and dried under reduced pressure over phosphorus pentoxide (1.25 g, 78%; m.p. 151-153°C); δ_H (250 MHz; $CDCl_3$/$CDCl_3$/$CD_3C\underline{H}_2$), 1.23 (3H, t, $C\underline{H}_3CH_2$), 1.10 and 1.41 (4H, m, cyclopropyl), 4.13 (2H, m, $CH_3C\underline{H}_2$), 4.43 (1H, s, CH); ν_{max} (nujol) 3490, 3464, 3380 (urea), 1700 (C-O), 1651 (NH-CO) cm^{-1}.

A solution of *n*-butyllithium in hexane (2.98 ml, 2.5M, 7.45 mmol) was added dropwise to diisopropylamine (0.98 ml, 6.77 mmol) in THF (40 ml) at -60°C. After stirring for 5 minutes, the foregoing urea-nitrile was quickly added as a solid. The solution was allowed slowly to warm to room temperature during which time it turned light yellow. This was stirred for two hours and then the solvent evaporated. The organic products were extracted into ethyl acetate from water. The aqueous layer was then acidified to pH = 1 using conc. HCl and re-extracted with ethyl acetate. The ethyl acetate layers were all combined, dried (Na_2SO_4) and the solvent removed to leave an oil. Addition of ether caused crystals of product to precipitate which were filtered and washed with chloroform (0.17 g, 36%); m.p. 191-193°C. δ_H (250 MHz; CD_3OD), 1.21 (3H, m, 3H of cyclopropyl), 1.68 (1H, m, 1H of cyclopropyl), 4.29 (1H, s, CH); ν_{max} (nujol) 3330, 3200 (NH-CO), 1727 (NH-CO); Found: C, 50.6; H, 4.4; N, 25.0 calc. for $C_7H_7N_3O_2$. C, 50.9; H, 4.2; N, 25.4%.

Hexahydro-2,6-dioxo-5-spirocyclopropylpyrimidine-6-carboxylic acid: **1** The foregoing nitrile **12** (5 mg) was dissolved in dilute hydrochloric acid (3M,2 ml) and the solution heated at 60°C for 9h. Evaporation of the solution under reduced pressure afforded the title compound as an amorphous white powder which was purified by

chromatography on silica eluting with dichloromethane/methanol (96:4 v/v) (3 mg, 54%). m.p.180°C (decomp.) Found: m/z (positive ion CI) 185.056831; $C_7H_9N_2O_4$ requires 185.56232. δ_H (250 MHz, CD_3OD) 1.19 (3H, m, H of cyclopropyl), 1.61 (1H, m, 1H of cyclopropyl), 3.48 (1H, s, H-6).

Acid-catalysed rearrangement of ***4*** *to* ***6****:* The ketone (**4**, 0.5g, 2.5 mmol) and *S*-α−methylbenzylamine (0.24 ml, 2.5 mmol) were dissolved in dry ether (1.5 ml) and a solution of titanium tetrachloride (0.19g, 1 mmol) in pentane (1 ml) added with stirring. The precipitate immediately formed was filtered off and the filtrate washed with water. The organic layer was separated, dried (Na_2SO_4) and the solvent removed under reduced pressure to leave an oil which was purified by chromatography on silica gel eluting with hexane/ethyl acetate (80:20 v/v). The product was characterised as a dihydrofuran **6** (0.3g, 60%). δ_H (90 MHz, $CDCl_3$) 1.30 (3H, t, CH_3CH_2), 2.17 (3H, s, MeCO), 2.35 (2H, t, CH_2=C), 3.60 (2H, t, CH_2O), 4.24 (2H, q, CH_3CH_2), 4.83 (2H, s, CH_2OAc). Found C: 54.1, H: 6.3% $C_{10}H_{14}O_5$ requires C: 56.1, H: 6.5%.

Ring expansion of ethyl 1-(1-acetyl)cyclopropane carboxylate: Ethyl 1-(1-acetyl)cyclopropane carboxylate[10] (0.85g, 5.9 mmol), benzylamine (0.84 ml, 6.5 mmol), benzene (5 ml), acetic acid (5 drops) and sodium sulphate (ca. 200 mg) were heated under reflux for 60 h. The solvent was evaporated under reduced pressure and the mixture of products separated by chromatography on silica gel eluting with hexane/ethyl acetate (80:20 v/v). Unreacted starting material (0.2g, 24%) and a new compound (0.7g, 48%) which was characterised as the dihydropyrrole **8.** δ_H (90 MHz, $CDCl_3$) 1.30 (3H, t, CH_3CH_2), 1.54 (3H, d, CH_3CHN), 2.13 (3H, s, MeC=C), 2.55-3.40 (4H, m, CH_2CH_2), 4.13 (2H, q, CH_3CH_2), 4.35 (1H, q, CHPh), 7.25 (5H, m, Ph). Found C: 74.1, H: 8.4, N: 5.1%. $C_{16}H_{21}NO_2$ requires C: 74.1, H: 8.2, N: 5.4%.

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