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Potential Wool Growth Inhibitors. Synthesis of DL-α-Amino-β-(5-hydroxy-4-oxo-3,4-dihydropyrimidin-2-yl)propionic Acid, a Pyrimidine Analogue of Mimosine

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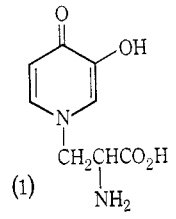
Abstract

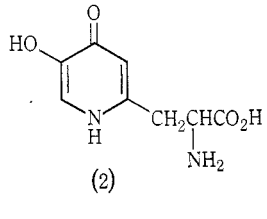
The synthesis of DL-α-amino-β-(5-hydroxy-4-oxo-3,4-dihydropyrimidin-2-yl)propionic acid (3), a pyrimidine analogue of mimosine, is described. The overall yield in four steps from aliphatic precursors is 25%.

Introduction

As part of a program directed towards the discovery and development of suitable 'chemical defleecing' agents, the synthesis of compounds related to the naturally occurring depilatory amino acid L-mimosine (1) has been undertaken.[1] Initially, synthetic routes to DL-mimosine, its homologues and an isomer (2) were examined. Preliminary investigations have shown that (2) is at least as active as (1) in *in vitro* and *in vivo* defleecing assays, and that essential features for activity in 4-pyridone-derived mimosine analogues included a hydroxy group α to the oxo function of the pyridone and an α-amino propionic acid side chain.[2] To extend this structure–activity survey, it was of interest to replace the 4-pyridone ring system with an analogously substituted 4-pyrimidinone. Therefore, the synthesis of the α-amino(pyrimidine)propionic acid (3) was undertaken.



(1)



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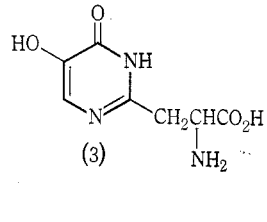


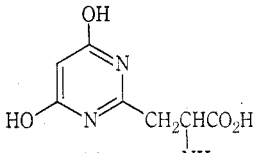
(3)

Discussion

It was initially proposed to synthesize (3) by methods successful with isomeric dioxygenated pyrimidinepropionic acids. For example, Springer, Haggerty and Cheng[3] prepared DL-α-amino-β-(4,6-dihydroxypyrimidin-2-yl)propionic acid (4) by condensation of the 2-methyl group of the bis(benzyloxy)pyrimidine (5) with ethyl oxalate to

[1] Harris, R. L. N., *Aust. J. Chem.*, 1976, **29**, 1329.
[2] Ward, K. A., and Harris, R. L. N., *Aust. J. Biol. Sci.*, in press.
[3] Springer, R. H., Haggerty, W. J., Jr, and Cheng, C. C., *J. Heterocycl. Chem.*, 1965, **2**, 49.

give the corresponding pyrimidinepyruvic ester, and subsequent elaboration of the amino acid side chain by oxime formation, hydrolysis and reduction. The benzyl-protecting groups were removed by hydrogenolysis.



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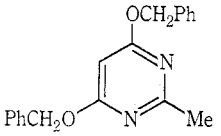
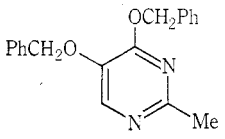
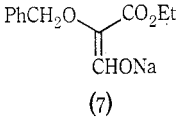


(5)

The bis(benzyloxy)pyrimidine (6), appropriate for the analogous synthesis of the amino acid (3), was prepared by methods similar to those used by Davoll and Laney[4] for related 5-benzyloxypyrimidines. Ethyl benzyloxyacetate (for which an improved method of preparation is given in the Experimental) was condensed with ethyl formate in the presence of sodium hydride to give the sodium salt of ethyl 1-benzyloxy-2-hydroxyacrylate (7). (This reaction takes place much more readily with sodium hydride than with sodium wire as used by Davoll and Laney.) Reaction of this intermediate *in situ* with acetamidine gave 5-benzyloxy-2-methylpyrimidin-4(3*H*)-one (8), which reacted with boiling $POCl_3$ to give the chloropyrimidine (9). It was found to be essential to boil the reaction mixture vigorously in this step to expel HCl as it was formed, otherwise extensive debenzylation occurred and yields of (9) were drastically reduced.



(6)



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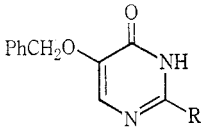
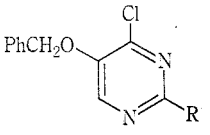


(8) R = Me
(10) R = CH_2OH



(9) R = Me
(11) R = CH_2Cl
(12) R = $CH_2C(CO_2Et)_2$ | NHAc

Conversion of (9) into the di(benzyloxy)pyrimidine (6) was readily achieved; however, the latter failed to condense with ethyl oxalate under conditions which had succeeded with its isomer (5). Apparently, electron-release from the benzyloxy group *para* to the 2-methyl group had deactivated this substituent sufficiently to prevent the condensation from taking place.

In another attempt to elaborate the amino acid side chain, the benzyloxypyrimidine (9) was treated with *N*-bromosuccinimide. It was hoped that halogenation of the methyl group would take place and the resulting bromomethyl pyrimidine could then be converted by reaction with diethyl acetamidomalonate and subsequent hydrolysis into the desired amino acid (3). This approach failed because bromination of (9) apparently occurred preferentially on the benzylic methylene group and benzaldehyde was liberated on workup.

A successful synthesis of (3) was finally devised, based on related pyrimidine amino acid syntheses of Shvachkin *et al.*[5] The hydroxymethylpyrimidinone (10) was prepared

[4] Davoll, J., and Laney, D. H., *J. Chem. Soc.*, 1956, 2124.
[5] Shvachkin, Yu. P., Syrtsova, L. A., and Prokof'ev, M. A., *Zh. Obshch. Khim.*, 1962, **32**, 2431.

by a method similar to that used for the pyrimidinone (8), with hydroxyacetamidine; in place of acetamidine. Reaction with $POCl_3$ gave the chloromethylpyrimidine (11);[6] again rapid expulsion of HCl as it formed was essential for an acceptable yield of the required product. Reaction of (11) with diethyl sodioacetamidomalonate in dry dimethylformamide gave the diester (12), which was hydrolysed by conc. HCl to the required pyrimidine amino acid (3) in an overall yield from ethyl benzyloxyacetate of 25%. The product crystallized from water as a stable monohydrate, as does DL-mimosine,[1,7] and gave a magenta colour with $FeCl_3$ in 1 N HCl and a yellow-brown colour with ninhydrin. The biological activity of (3) is currently being evaluated and will be described elsewhere.

Experimental

Melting points are uncorrected and were taken on a Büchi melting point apparatus. N.m.r. were recorded on a Varian A-60 instrument, tetramethylsilane [in $CDCl_3$ or $(CD_3)_2SO$] or trimethylsilylpropanesulphonic acid (in CF_3COOD–D_2O) being used as internal reference. Microanalyses were performed by the Australian Microanalytical Service.

Ethyl Benzyloxyacetate

Sodium hydride–oil suspension (60% NaH, 160 g, 4 mol) was suspended in dry benzene (2 l.) and benzyl alcohol (432 g, 4 mol) was added slowly during 1½ h with stirring and protection from moisture. The bulky grey suspension of sodium benzylate was stirred a further 1 h; a solution of chloroacetic acid (188 g, 2 mol) in benzene (1 l.) was then added during 1 h with stirring at a rate such that the mixture gently refluxed. Reflux was continued for 1 h after the addition and the mixture left overnight at room temperature. Water (1 l.) was added and the mixture stirred until all solids had dissolved. The two phases were filtered through Celite; the benzene layer was separated and washed with water (2 × 200 ml). The combined aqueous layers were acidified (pH 2) with conc. HCl and the heavy oil that precipitated was extracted into methylene chloride (2 × 500 ml). The combined methylene chloride extracts were washed with water, dried ($MgSO_4$) and the solvent removed in vacuum to give benzyloxyacetic acid (321 g, 98%) as a viscous oil. The acid (321 g), absolute ethanol (400 ml), benzene (500 ml) and conc. H_2SO_4 (32 ml) were heated together under reflux for 3 h. The mixture was washed with water, then with 5% aqueous sodium bicarbonate solution, until the washings were alkaline. The organic phase was dried ($MgSO_4$) and distilled in vacuum to give ethyl benzyloxyacetate as a colourless oil (305 g, 85%), b.p. 144–148°/12 mm (lit.[8] 180°/15 mm).

5-Benzyloxy-2-methylpyrimidin-4(3H)-one (8)

Sodium hydride–oil suspension (60% NaH, 12·5 g, 0·31 mol) was added to a solution of ethyl benzyloxyacetate (48·5 g, 0·25 mol) and ethyl formate (20 ml) in dry ether (100 ml). A condenser was fitted to the flask and the vigorous reaction allowed to proceed at room temperature for 30 min. Most of the ether was removed in vacuum at 30°, leaving a bulky mass of the sodium salt of ethyl 1-benzyloxy-2-hydroxyacrylate (7).[4]

Acetamidine hydrochloride (23·75 g, 0·25 mol) was dissolved in ethanol (250 ml) containing sodium ethoxide by solution of sodium (5·8 g, 0·25 mol) and the filtered ethanolic solution of acetamidine added to the resulting sodium enolate. The mixture was refluxed for 2 h and most of the ethanol removed in vacuum. The residue was dissolved in water (250 ml); the solution was treated with charcoal, filtered through Celite and acidified (pH 5) with acetic acid. The crude product (37 g) which precipitated was washed with a little acetone and recrystallized from ethyl acetate to give *5-benzyloxy-2-methylpyrimidin-4(3H)-one* as colourless needles (30 g, 56%), m.p. 186–189° (Found: C, 66·7; H, 5·7; N, 13·1. $C_{12}H_{12}N_2O_2$ requires C, 66·7; H, 5·6; N, 13·0%). N.m.r. ($CDCl_3$) δ 2·4 (s, 3H, CH_3), 5·1 (s, 2H, $ArCH_2O$) and 7·2–7·6 (m, 6H, aromatic protons overlapping H 6).

[6] Klarer, W., and Urech, E., *Helv. Chim. Acta*, 1944, **27**, 1762.
[7] Spenser, I. D., and Notation, A. D., *Can. J. Chem.*, 1962, **40**, 1374.
[8] Rothstein, B., *Bull. Soc. Chim. Fr.*, 1932, **51**, 691.

5-Benzyloxy-4-chloro-2-methylpyrimidine (9)

The foregoing pyrimidine (20 g) was heated vigorously under reflux with phosphorus oxychloride (50 ml) for 1 h. Excess $POCl_3$ was removed in vacuum and ice water added to the residue. The solid product was collected and recrystallized from aqueous ethanol to give *5-benzyloxy-4-chloro-2-methylpyrimidine* as colourless needles (17 g, 69%), m.p. 96–99° (Found: C, 61·3; H, 4·9; N, 11·9. $C_{12}H_{11}ClN_2O$ requires C, 61·4; H, 4·7; N, 11·9%). N.m.r. ($CDCl_3$) δ 2·6 (s, 3H, CH_3), 5·2 (s, 2H, $ArCH_2O$), 7·4 (s, 5H, C_6H_5) and 8·2 (s, 1H, H 6). If the reaction mixture was not vigorously boiled to expel the HCl generated, appreciable cleavage of the benzyl ether occurred and the yield was drastically reduced.

4,5-Bis(benzyloxy)-2-methylpyrimidine (6)

The foregoing chloropyrimidine (11·7 g) was added to a solution of sodium benzylate prepared from sodium hydride–oil suspension (60% NaH, 4 g) and benzyl alcohol (5·4 g) in dry benzene (100 ml). The mixture was refluxed with stirring for 4 h, washed with water, dried ($MgSO_4$) and evaporated in vacuum. The crude product was recrystallized from light petroleum (b.p. 60–80°) to give *4,5-bis-(benzyloxy)-2-methylpyrimidine* as colourless needles (13 g, 85%), m.p. 53–54° (Found: C, 74·6; H, 6·0; N, 9·0. $C_{19}H_{18}N_2O_2$ requires C, 74·5; H, 5·9; N, 9·2%). N.m.r. ($CDCl_3$) δ 2·5 (s, 3H, CH_3), 5·05 and 5·5 (each s, 2H, $ArCH_2O$), 7·1–7·55 (m, 10H, C_6H_5) and 7·9 (s, 1H, H 6).

Attempts to condense the 4,5-dibenzyloxypyrimidine with ethyl oxalate under conditions similar to those used by Springer, Haggerty and Cheng[3] with 4,6-dibenzyloxy-2-methylpyrimidine were unsuccessful and starting material was recovered unchanged.

Attempted Bromination of (9)

The pyrimidine (9) (0·8 g, 0·03 mol) and *N*-bromosuccinimide (0·6 g, 0·03 mol) were heated under reflux in carbon tetrachloride (50 ml) with illumination from a 75-W tungsten lamp for 1 h. The solution was allowed to cool, filtered and the solvent removed in vacuum. The residual oil (1·1 g) could not be distilled in vacuum without decomposition. The n.m.r. ($CDCl_3$) of the crude material showed no signal in the $ArCH_2O$ region and the signal due to the CH_3 group was still present (δ 2·75). The pyrimidine CH had shifted downfield and now appeared as two doublets (δ 8·8 and 9·0). When the crude bromo compound was dissolved in methanol and basified with 10% NaOH, benzaldehyde was immediately liberated. It was identified by n.m.r. and as the dinitrophenylhydrazone (m.p., mixed m.p. 237°).

5-Benzyloxy-2-hydroxymethylpyrimidin-4(3H)-one (10)

Ethyl benzyloxyacetate (32·5 g, 0·15 mol), sodium hydride (60% suspension in oil, 7·5 g, 0·19 mol) and ethyl formate (12 ml) were allowed to react in ether, as described above. After removal of the ether, a solution of hydroxyacetamidine from the hydrochloride[6] (18·4 g, 0·17 mol) and sodium ethoxide (11·6 g, 0·17 mol) in ethanol (150 ml) was added. The mixture was refluxed with stirring for 2 h, poured into water (300 ml), treated with charcoal and filtered through Celite. On acidification with HCl to pH 3 a heavy oil appeared and rapidly crystallized. The mixture was kept overnight at 5°, the product collected and washed with water and ether. On crystallization from ethyl acetate *5-benzyloxy-2-hydroxymethylpyrimidin-4(3H)-one* was obtained as colourless needles (19 g, 55%), m.p. 148–149° (Found: C, 60·0; H, 5·2; N, 11·5. $C_{12}H_{12}N_2O_3,\frac{1}{2}H_2O$ requires C, 59·7; H, 5·4; N, 11·6%). N.m.r. [$(CD_3)_2SO$] δ 3·3 (broad exchangeable signal, OH and NH), 4·2 (s, 2H, CH_2), 5·0 (s, 2H, $ArCH_2O$), 7·35 (s, 5H, C_6H_5) and 7·5 (s, 1H, H 6).

5-Benzyloxy-4-chloro-2-chloromethylpyrimidine (11)

The foregoing hydroxymethylpyrimidinone (13·5 g) was heated *vigorously* under reflux with $POCl_3$ (50 ml) for 15 min. Excess $POCl_3$ was removed in vacuum and ice water added to the residue. The solid product was collected and recrystallized (charcoal) from aqueous methanol to give *5-benzyloxy-4-chloro-2-chloromethylpyrimidine* (10·6 g, 68%) as colourless flat needles, m.p. 109–111° (Found: C, 53·4; H, 3·9; N, 10·3. $C_{12}H_{10}Cl_2N_2O$ requires C, 53·5; H, 3·75; N, 10·3%). N.m.r. ($CDCl_3$) δ 4·65 (s, 2H, CH_2Cl), 5·25 (s, $ArCH_2O$), 7·4 (s, 5H, ArH), 8·3 (s, 1H, H 6). When the reaction mixture was not vigorously refluxed to drive out HCl formed, the yield was much lower due to cleavage of the benzyl ether (benzyl chloride was formed).

Ethyl α-Acetamido-β-(5-benzyloxy-4-chloropyrimidin-2-yl)-α-ethoxycarbonylpropionate (12)

Sodium hydride–oil suspension (60%, 2 g, 0·05 mol) was added in portions to a solution of diethyl acetamidomalonate (9·6 g, 0·04 mol) in dry dimethylformamide (50 ml). After effervescence of hydrogen had ceased, 5-benzyloxy-4-chloro-2-chloromethylpyrimidine (10 g, 0·04 mol) was added and the mixture heated on a steam bath with stirring for 2 h. The mixture was poured into water (1 l.) and the product extracted into ethyl acetate (3 × 150 ml). The extracts were combined, dried ($MgSO_4$), treated with charcoal and evaporated. The residue was triturated with benzene–light petroleum (b.p. 60–80°) (1 : 1) to give *ethyl α-acetamido-β-(5-benzyloxy-4-chloropyrimidin-2-yl)-α-ethoxycarbonylpropionate* as buff prisms (13 g, 74%), which crystallized from benzene–light petroleum with m.p. 121–122° (Found: C, 56·4; H, 5·5; N, 9·4. $C_{21}H_{24}ClN_3O_6$ requires C, 56·1; H, 5·4; N, 9·3%). N.m.r. ($CDCl_3$) δ 1·25 (t, 6H, OCH_2CH_3), 1·9 (s, 3H, $COCH_3$), 3·95 (s, 2H, CH_2–C), 4·3 (q, 4H, OCH_2CH_3), 5·2 (s, 2H, $ArCH_2O$), 6·7 (s, exchangeable, 1H, NH), 7·4 (s, 5H, C_6H_5) and 8·2 (s, 1H, H6).

DL-*α-Amino-β-(5-hydroxy-4-oxo-3,4-dihydropyrimidin-2-yl)propionic Acid (3)*

The foregoing pyrimidinepropionate (13) (8 g) was heated under reflux in conc. HCl (80 ml) for 20 h. Excess acid was removed in vacuum, the residue dissolved in water (20 ml), the solution treated with charcoal, filtered and neutralized (pH 5) with conc. ammonia. The product rapidly crystallized and was collected after keeping overnight at 5°, washed with 50% aqueous acetone and dried at 85°. DL-*α-Amino-β-(5-hydroxy-4-oxo-3,4-dihydropyrimidin-2-yl)propionic acid* was obtained as pale yellow prisms (3·3 g, 92%), m.p. 220–225°, and analysed as a monohydrate (Found: C, 38·3; H, 5·3; N, 19·1. $C_7H_9N_3O_4,H_2O$ requires C, 38·7; H, 5·1; N, 19·4%). It gave a magenta colour with ferric chloride in 1 N HCl and a yellow-brown colour with ninhydrin. N.m.r. (20% CF_3COOD–D_2O) δ 3·65 (d, 2H, CH_2CH), 4·7 (t, 1H, CH_2CH) and 7·7 (s, 1H, H6) (water peak 5·55).

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