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(54) PYRAZOLO (3,4 *d*) PYRIMIDINES

(71) We, THE WELLCOME FOUNDATION LIMITED, of 183—193 Euston Road, London, N.W.1., a company incorporated in England do hereby declare the invention which was communicated from BURROUGHS WELLCOME & Co. (U.S.A.) INC., of 1, Scarsdale Road, Tuckahoe, New York 10707, a Company Incorporated in the State of New York, United States of America, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a method of synthesising pyrazolo (3,4-*d*) pyrimidines.

4-Hydroxypyrazolo (3,4-*d*) pyrimidine, which is also known as allopurinol, is active as an inhibitor of the enzyme xanthine oxidase which catalyzes the oxidation of most purines to uric acid in vivo. Alopurlinol has thus been found to relive the symptons of gout since it diminishes greatly the amount of uric acid formed. In the same manner, it slows down the oxidation of 6-mercaptopurine to 6-thiouric acid, thus permitting smaller doses of 6-mercaptopurine to be employed.

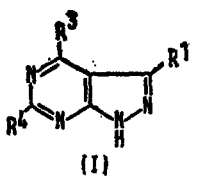
Allopurinol is administered to patients in relatively large doses generally in the range of 100 to 800 mg. per person per day, but sometimes in still larger doses. Also, allopurinol is given over protracted periods.

The purity of the product is thus of even greater importance than with most pharmaceuticals. Some contaminants have been found difficult and expensive to remove from the final product. It is consequently of great practical importance that a method of synthesis should not only be quantitatively efficient but also that the final product should be satisfactorily pure.

Examples of other pyrazolo (3,4-*d*) pyrimidines possessing valuable xanthine oxidase inhibiting activity are 4,6-dihydroxy-pyrazolo (3,4-*d*) pyrimidine, also known as oxypurinol, and 4-mercapto-pyrazolo (3,4-*d*) pyrimidine, also known as thiopurinol.

A number of methods of synthesis are known for pyrazolo (3,4-*d*) pyrimidines such as those described above. For example, allopurinol may be prepared by reacting ethoxymethylenemalonodinitrile with hydrazine to give 3-amino-4-cyanopyrazole, hydrolysing the 3-amino-4-cayonpyrazole to 3-amino-4-carboxamidopyrazole, and reacting the 3-amino-4-carboxamidopyrazole with formamide and/or formic acid. Oxypurinol may be prepared by a similar synthetic route in which urea is used instead of formamide and/or formic acid.

It has now been found that certain pyrazolo (3,4-*d*) pyrimidines, including those mentioned above, can be prepared by a new synthetic route. Thus, according to the present invention there is provided a method of preparing a compound of formula (I):



(I)

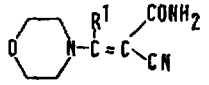
(I)

wherein

R^1 is a hydrogen atom or an alkyl group having 1 to 4 carbon atoms,

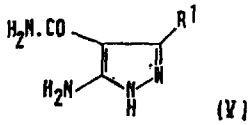
R^3 is a hydroxy group or a mercapto group, and

R^4 is a hydrogen atom or a hydroxy group, provided that R^4 is not a hydroxy group when R^3 is a mercapto group, which comprises reacting a compound of formula (III):



(III)

with hydrazine to produce a compound of formula (V)



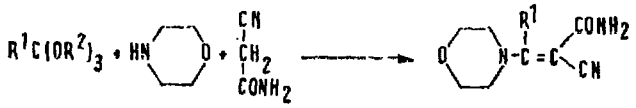
(V)

and reacting a compound of formula (V) with urea or with formamide and/or formic acid to produce a compound of formula (I).

The reaction of a compound of formula (III) with hydrazine is conveniently carried out in the presence of heat. The compound of formula (V) produced by the reaction is a base but, if it is to be isolated, it is preferably converted by reaction with an acid into a salt, desirably a salt of a pharmaceutically acceptable acid such as sulphuric acid.

A compound of formula (I) so produced by the complete sequence can be converted to a different compound of formula (I) by standard methods. For example, 4-hydroxy-pyrazolo (3,4-*d*) pyrimidine can be converted to 4-mercaptopyrazolo (3,4-*d*) pyrimidine by reaction with a sulphide such as phosphorus pentasulphide. When a 3-alkylpyrazolo (3,4-*d*)-pyrimidine of formula (I) is produced by the method of the present invention, the 3-alkyl group can be removed by oxidation to a carboxy group followed by decarboxylation of this group.

It has also been found, as claimed in the Specification of copending patent application No. 29533/71 (Serial No. 1252436) that a compound of formula (III) can be prepared by reacting a compound $R^1C(OR^2)_3$, wherein R^2 is an alkyl group having from 1 to 4 carbon atoms and is preferably an ethyl group, with cyanoacetamide and morpholine according to the reaction scheme:



(III)

In this method, the compound $R^1C(OR^2)_3$ and the morpholine are each advantageously employed in a slight excess, for example, about 20% excess. Also, a moderately volatile polar solvent is desirable and serves both to moderate the reaction and to improve the qualtity of the crystalline product. Acetonitrile is the preferred solvent, but an alcohol having from 1 to 4 carbon atoms, such as butanol or isopropanol can be used. An alcohol R^2OH is formed in the course of the reaction and is consequently always present in the solvent from which the product crystallizes. The morpholine is both a reagent and a catalyst. The starting materials in the preparation of a compound of formula (III) are preferably mixed together and then heated to reflux, preferably at a temperature from 60°C to 120°C.

A number of other amines, such as aniline, substituted anilines, piperidine and benzylamine, act in a formally similar manner to morpholine, for example, 3-anilino-2-cyanoacrylamides are known compounds. For the present purpose, however, the stronger bases, such as piperidine and benzylamine, and the anilines suffer from serious disadvantages. For example, the anilines give "analytically" pure products at all stages

but which are deeply coloured. Removal of these coloured contaminants is prohibitively expensive. The disadvantage of the stronger bases, such as piperidine, is that they also catalyse reactions of cyanoacetamide with itself to form pyridine derivatives, which are difficult to remove and are intolerable in the final compound. Morpholine (pK_a, c. 8.3), however, has the advantage in that its weak basicity makes it a poor catalyst in the self-condensation of cyanoacetamide. Morpholine also has the advantage that it lacks the aromatic reactivity of the anilines and thus coloured by-products, which are probably polymeric, are not formed. Additionally, morpholine has the advantage that the compound of formula III) has favourable physical properties and can be isolated readily in good yield and in high purity. Thus, an entire reaction sequence comprising the preparation of a compound of formula (III) and its conversion as described above to a compound (I) can be carried out without recrystallization of any of the intermediates being necessary.

The method of the present invention is particularly preferred when R^1 is a hydrogen atom because the end products, i.e., the compounds of formula (I) are the important pyrazolo (3,4-*d*) pyrimidines, allopurinol, oxypurinol and thiopyrinol. It has been found that the compound of formula (I) is produced in a very high state of purity by the present method and also in very good yield. As already stated herein, purity of product is very important in a preparative route to a pharmaceutical such as allopurinol.

Thus in a preferred aspect there is provided a method of preparing 4-hydroxypyrazolo (3,4-*d*) pyrimidine in which 3-morpholino-2-cyanoacrylamide is reacted with hydrazine to produce 3-amino-4-carboxamidopyrazole, and the 3-amino-4-carboxamidopyrazole is reacted with formamide and/or formic acid. In another aspect 4,6-dihydroxypyrazolo (3,4-*d*) pyrimidine is prepared by a similar synthetic route in which urea is used instead of formamide and/or formic acid in the final stage.

In a further aspect, the present invention provides a method of preparing a compound of formula (V) or a salt thereof.

The following Examples 1, 4, 5 and 6 illustrate the present invention and examples 2 and 3 describe the preparation of intermediates.

EXAMPLE 1

(a) 3-Morpholino-2-cyanoacrylamide

A stirred mixture of cyanoacetamide (63 g.), triethylorthoformate (134 g.), morpholine (82.5 g.) and acetonitrile (37.5 ml.) was heated under reflux for 4 hours. The initial reflux temperature was 117°C and the final reflux temperature was 82°C.

At the end of the reflux period the mixture was cooled to 30°C and the heavy crystalline precipitate was collected and washed with 2×75 ml. of ethanol. The product was dried *in vacuo* at 30°C. Wt.=111 g. Yield=82% m.p. 173—175°C.

(b) 3-Aminopyrazole-4-carboxamide hemi-sulphate

To water (253 ml.) at 60°C was added 3-morpholino-2-cyanoacrylamide (63.4 g.) and 85% technical hydrazine hydrate (22.7 g.). The mixture was rapidly heated to 95°C and the temperature was maintained at >90°C for 20 minutes. The mixture was then cooled to 60°C and the pH carefully adjusted to 1.5 by the addition of a mixture of sulphuric acid (45.7 g.) and ice (45.7 g.). The acidified reaction mixture was cooled to 5°C and the crystalline product collected and washed with cold water (2×100 ml.) and acetone (2×50 ml.). The product was dried *in vacuo* at 80°C. Wt=5.8 g. Yield=95% m.p. 237—239°C.

(c) 4-Hydroxypyrazolo (3,4-*d*) pyrimidine

A suspension of 3-aminopyrazole-4-carboxamide hemi-sulphate (113 g.) in formamide (325 ml.) was stirred and heated to 145°C. The reaction was held at 145°C for 5 hours. The reaction mixture was then cooled to 30°C and the product collected and washed with formamide (2×50 ml.), water (2×150 ml.) and acetone (2×100 ml.). Wt. of crude product=79 gms. The crude product was recrystallized by dissolution in a solution made from sodium hydroxide (25 g.) in water (1200 ml.) with treatment at 25°C with charcoal (8 g.), followed by reprecipitation by the addition of concentrated hydrochloric acid to pH 5. The product was collected and washed with cold water (2×300 ml.), acetone (2×200 ml.) and dried *in vacuo* at 60°C. Weight=70 g. Yield=80%.

EXAMPLE 2

3-Methyl-3-morpholino-2-cyanoacrylamide

A stirred mixture of cyanoacetamide (84 g.), triethylorthoacetate (178 g.) and

morpholine (108 g.) was heated under reflux for 3 hours. The initial reflux temperature was 104°C and the final reflux temperature was 89°C. At the end of the reflux period acetonitrile (50 ml.) was added, the mixture was cooled to 25°C, the crystalline precipitate was collected, and washed with 2×100 ml. of cold ethanol. Weight= 104.7 g. Yield=53%. A sample was recrystallized from ethanol as white needles m.p. 173—174.5°C.

EXAMPLE 3

3-Ethyl-3-morpholino-2-cyanoacrylamide

A stirred mixture of cyanoacetamide (84 g.) triethylorthopropionate (193 g.) and morpholine (108 g.) was heated under reflux for 3 hours. The initial reflux temperature was 121°C and the final reflux temperature was 91°C. At the end of the reflux period acetonitrile (50 ml.) was added, the mixture was cooled to 25°C, the crystalline precipitate was collected, and washed with 2×100 ml. of cold ethanol. Weight= 87 g. Yield=42%. A sample was recrystallized from ethanol as white needles m.p. 158°C.

EXAMPLE 4

(a) 3-Morpholino-2-cyanoacrylamide

Cyanoacetamide (67.4 Kg.), triethylorthoformate (143.9 Kg.), morpholine (88.2 Kg.) and acetonitrile (33.0 Kg.) were placed in a suitable 100 gallon reactor equipped with a stirrer and a reflux condenser. The batch was heated to reflux and the refluxing continued for 4 hours.

The initial reflux temperature should be above 100°C, and the final reflux temperature should be approximately 82°C. In large scale operations such as this it is preferable to seed the batch periodically, and more preferably about every 30 minutes, with 3-morpholino-2-cyanoacrylamide after initial reflux until copious precipitation is obvious.

After completion of the reflux period, the batch was cooled rapidly to 30°C±1°C and centrifuged. The wash was a blend of ethanol (74.9 Kg.), water (10.7 litres) and ice (10.7 Kg.). The 3-morpholino-2-cyanoacrylamide was vacuum dried at 30°C.

(b) 3-Aminopyrazole-4-carboxamide hemi-sulfate

Water (210.5 litres) was added to a reactor heated to 70°C. 3-morpholino-2-cyanoacrylamide (52.8 Kg) was added and the temperature readjusted to 70°C. The jacket round the reactor was drained, and 85% hydrazine hydrate (18.1 Kg.) was added to the reactor. The heat of the reaction caused the temperature to increase to 95 to 100°C over a 5 minute period. Ten minutes after the hydrazine addition, the reaction mixture was cooled to 55 to 60°C and a mixture of sulfuric acid (37.5 Kg.) and ice (37.5 Kg.) was carefully added to pH 1.5±0.2. The batch was cooled to 0 to 5°C, and centrifuged. It was washed with water (66.6 litres).

The exact quantity of acid required may vary since there is some loss of excess hydrazine and evolved-morpholine during the course of the reaction.

(c) 4-Hydroxypyrazole (3,4-*d*) pyrimidine

Formamide (244.8 Kg.) and 3-amino-pyrazole-4-carboxamide hemi-sulphate (71.6 Kg.) were charged to a well vented reactor. The mixture was heated to 140 to 145°C, and held at this temperature for five hours. It was then cooled to 5 to 10°C, and centrifuged. The cake was rinsed with formamide (50.6 Kg.), followed by water (89.2 Kg.).

EXAMPLE 5

(a) 3-Amino-5-ethylpyrazole-4-carboxamide

3-Ethyl-3-morpholino-2-cyanoacrylamide (52.25 g.), water (200 ml.) and 85% hydrazine hydrate (15 ml.) were stirred together at room temperature for one (1) hour and then heated to 45—60°C for 15 minutes. The reaction mixture was then cooled to 5°C and the product collected, washed with acetone, and dried *in vacuo* at 50°C. Wt.=31.7 g. m.p. 190—191°C after recrystallisation from water. $C_6H_{10}N_4O$ requires C, 46.74; H, 6.53; N, 36.34. Found C, 46.64; H, 6.53; N, 36.46%.

(b) 3-Ethyl-4-hydroxypyrazolo (3,4-*d*) pyrimidine

3-Amino-5-ethylpyrazole-4-carboxamide (18.5 g.) and formamide (64.15 ml.) were heated together at 140°C for 5 hours. The reaction mixture was then cooled to 5°C, the product collected, and washed with formamide (20 ml.) and water (50 ml.). The product was then dissolved in a solution of sodium hydroxide (45 g.) in water

(180 ml.) and the solution was treated at room temperature with charcoal (Darco G-60) (Registered Trade Mark) (3.6 g.). The filtrate, after removal of charcoal, was heated to 60°C and the pH was at that temperature adjusted to 5 by the addition of glacial acetic acid. The mixture was cooled to 5°C and the pure product was collected, washed with water (200 ml.) and dried *in vacuo* at 60°C. Wt.=11.7 g. m.p. 275—276.5°C. $C_7H_8N_4O$ requires C, 50.92; H, 4.88; N, 33.90. Found C, 50.54; H, 4.89; N, 33.86%.

EXAMPLE 6

(a) 3-n-Butyl-3-morpholino-2-cyanoacrylamide

Cyanoacetamide (10.6 g.), trimethylorthovalerate (24.6 g.) and morpholine (13.8 ml.) were heated together at reflux temperature (120°C→76°C) for 2½ hours. The reaction mixture was cooled and the crystalline product collected and washed with ethanol. Wt.=13.5 g. m.p. 185—187°C after recrystallisation from ethanol $C_{12}H_{19}N_3O_2$ requires C, 60.85; H, 8.02; N, 17.70. Found C, 60.85; H, 8.25; N, 17.30%.

(b) 3-Amino-5-n-butylpyrazole-4-carboxamide

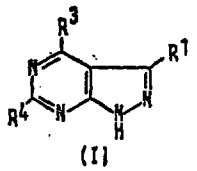
3-n-butyl-3-morpholino-2-cyanoacrylamide (23.7 g.), water (72.5 ml.) and 85% hydrazine hydrate (5.9 ml.) were heated together at 60°C for one (1) hour by which time the starting material was completely consumed as judged by U.V. spectroscopy. The reaction mixture was cooled to 5°C and the crystalline product collected, washed with water and dried. Wt.=13.4 g. m.p. 160—162°C after recrystallisation from water. $C_8H_{14}N_4O$ requires C, 52.75; H, 7.76; N, 30.74. Found C, 52.53; H, 7.85; N, 30.79%.

(c) 3-n-Butyl-4-hydroxypyrazolo (3,4-*d*) pyrimidine

3-amino-5-n-butylpyrazole-4-carboxamide (12.7 g.) and formamide (38 ml.) were heated together at 140°C for 5 hours. The reaction mixture was then cooled to 5°C, the product collected and washed with cold formamide (15 ml.). The filter cake was dissolved in water (150 ml.) and 10 N sodium hydroxide (3.1 ml.) and the solution was treated with charcoal (Darco G-60; 1 g.) at room temperature. The filtrate, after charcoal removal, was heated to 60°C and the pH adjusted to 5.5 using glacial acetic acid. The slurry was cooled to 5°C and the pure product collected, washed with water and dried *in vacuo* at 50°C. Wt.=11.4 g. m.p. 236—238°C. $C_9H_{12}N_4O$ requires C, 56.25; H, 6.30; N, 29.20. Found C, 56.05; H, 6.45; N, 29.32%.

WHAT WE CLAIM IS:—

1. A method of preparing a compound of formula (I)

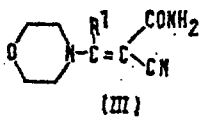


(I)

wherein R^1 is a hydrogen atom or an alkyl group having from 1 to 4 carbon atoms,

R^3 is a hydroxy group or a mercapto group, and

R^4 is a hydrogen atom or a hydroxy group, provided that R^4 is not a hydroxy group when R^3 is a mercapto group, which comprises reacting a compound of formula (III)



(III)

(180 ml.) and the solution was treated at room temperature with charcoal (Darco G-60) (Registered Trade Mark) (3.6 g.). The filtrate, after removal of charcoal, was heated to 60°C and the pH was at that temperature adjusted to 5 by the addition of glacial acetic acid. The mixture was cooled to 5°C and the pure product was collected, washed with water (200 ml.) and dried *in vacuo* at 60°C. Wt.=11.7 g. m.p. 275—276.5°C. $C_7H_8N_4O$ requires C, 50.92; H, 4.88; N, 33.90. Found C, 50.54; H, 4.89; N, 33.86%.

EXAMPLE 6

(a) 3-n-Butyl-3-morpholino-2-cyanoacrylamide

Cyanoacetamide (10.6 g.), trimethylorthovalerate (24.6 g.) and morpholine (13.8 ml.) were heated together at reflux temperature (120°C→76°C) for 2½ hours. The reaction mixture was cooled and the crystalline product collected and washed with ethanol. Wt.=13.5 g. m.p. 185—187°C after recrystallisation from ethanol $C_{12}H_{19}N_3O_2$ requires C, 60.85; H, 8.02; N, 17.70. Found C, 60.85; H, 8.25; N, 17.30%.

(b) 3-Amino-5-n-butylpyrazole-4-carboxamide

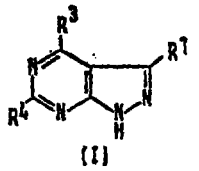
3-n-butyl-3-morpholino-2-cyanoacrylamide (23.7 g.), water (72.5 ml.) and 85% hydrazine hydrate (5.9 ml.) were heated together at 60°C for one (1) hour by which time the starting material was completely consumed as judged by U.V. spectroscopy. The reaction mixture was cooled to 5°C and the crystalline product collected, washed with water and dried. Wt.=13.4 g. m.p. 160—162°C after recrystallisation from water. $C_8H_{14}N_4O$ requires C, 52.75; H, 7.76; N, 30.74. Found C, 52.53; H, 7.85; N, 30.79%.

(c) 3-n-Butyl-4-hydroxypyrazolo (3,4-*d*) pyrimidine

3-amino-5-n-butylpyrazole-4-carboxamide (12.7 g.) and formamide (38 ml.) were heated together at 140°C for 5 hours. The reaction mixture was then cooled to 5°C, the product collected and washed with cold formamide (15 ml.). The filter cake was dissolved in water (150 ml.) and 10 N sodium hydroxide (3.1 ml.) and the solution was treated with charcoal (Darco G-60; 1 g.) at room temperature. The filtrate, after charcoal removal, was heated to 60°C and the pH adjusted to 5.5 using glacial acetic acid. The slurry was cooled to 5°C and the pure product collected, washed with water and dried *in vacuo* at 50°C. Wt.=11.4 g. m.p. 236—238°C. $C_6H_{12}N_4O$ requires C, 56.25; H, 6.30; N, 29.20. Found C, 56.05; H, 6.45; N, 29.32%.

WHAT WE CLAIM IS:—

1. A method of preparing a compound of formula (I)

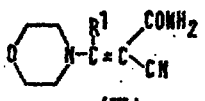


(I)

wherein R^1 is a hydrogen atom or an alkyl group having from 1 to 4 carbon atoms,

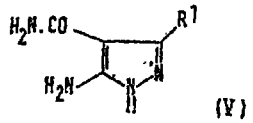
R^3 is a hydroxy group or a mercapto group, and

R^4 is a hydrogen atom or a hydroxy group, provided that R^4 is not a hydroxy group when R^3 is a mercapto group, which comprises reacting a compound of formula (III)



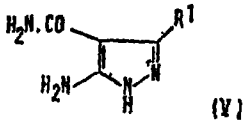
(III)

with hydrazine to produce a compound of formula (V)



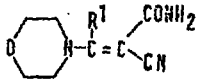
(V)

and reacting a compound of formula (V) with urea or with formamide and/or formic acid to produce a compound of formula (I), and optionally converting a compound of formula (I) so produced into a different compound of formula (I) by standard methods.



(V)

wherein R^1 is defined as in claim 1, or a salt thereof, which comprises reacting a compound of formula (III) with hydrazine



(III)

wherein R^1 is defined as in claim 1, and if necessary, converting the base into the salt by reaction with an appropriate acid.

3. A method according to claim 1, in which the compound of formula (V) is not recrystallised.

4. A method according to any of the preceding claims, in which R^1 is a hydrogen atom.

5. A method according to any of the preceding claims, in which the reaction of a compound of formula (III) with hydrazine is carried out in the presence of heat.

6. A method according to any of the preceding claims, in which a compound of formula (V) is isolated in the form of a salt of a pharmaceutically acceptable acid.

7. A method of preparing 4-hydroxypyrazolo (3,4-*d*) pyrimidine, in which 3-morpholino-2-cyanoacrylamide is reacted with hydrazine to produce 3-amino-4-carboxamidopyrazole, and the 3-amino-4-carboxamidopyrazole is reacted with formamide and/or formic acid.

8. A method of preparing 4,6-dihydroxypyrazolo (3,4-*d*) pyrimidine, in which 3-morpholino-2-cyanoacrylamide is reacted with hydrazine to produce 3-amino-4-carboxamidopyrazole, and the 3-amino-4-carboxamidopyrazole is reacted with urea.

9. A method of preparing 4-mercaptopyrazolo (3,4-*d*)pyrimidine, in which 4-hydroxypyrazolo (3,4-*d*) pyrimidine, when prepared according to claim 7, is reacted with phosphorus pentasulphide.

10. A method of preparing a compound of formula (I) as claimed in claim 1, substantially as hereindescribed with reference to any one of Examples 1, 4, 5 and 6.

11. A method of preparing a compound of formula (V) as claimed in claim 2, substantially as herein described with reference to any one of Examples 1, 4, 5 and 6.

12. A compound of formula (I) as defined in claim 1, whenever prepared according to a method claimed in any of claims 1 and 3 to 10.

13. A compound of formula (V) as defined in claim 2, whenever prepared according to a method claimed in any of claims 1 to 11.

14. 4-Hydroxypyrazolo (3,4-*d*) pyrimidine, whenever prepared according to a method claimed in any of claims 1 and 3 to 7, 9 and 10.

15. 4,6-Dihydroxypyrazolo (3,4-*d*) pyrimidine, whenever prepared according to a method claimed in any of claims 1 and 3 to 6, 8 and 10.

16. 4-Mercaptopyrazolo (3,4-*d*) pyrimidine, whenever prepared according to a method claimed in any of claims 1 and 3 to 6, 9 and 10.

M. P. JACKSON,
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