

PATENT SPECIFICATION

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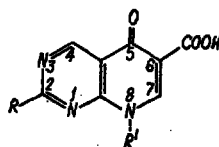
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(54) 8-ALKYL-5-OXO-5,8-DIHYDRO-PYRIDO(2,3-d) PYRIMIDINE-6-CARBOXYLIC ACIDS AND THEIR PREPARATION

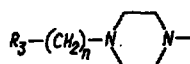
(71) We, LABORATOIRE ROGER BELLON, a French Body Corporate of 159, Avenue des Champs-Élysées, 92200 Neuilly-sur-Seine, France, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention provides a new process for the preparation of 8-alkyl-5-oxo-5,8-dihydropyrido(2,3-d)pyrimidine-6-carboxylic acids of the formula



I

in which R is 4-hydroxypiperidino, piperazino or N-substituted piperazino of the formula:

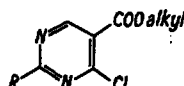


where n is 0, 1, 2 or 3, and R₃ is hydrogen, hydroxyl, vinyl, phenyl, or phenyl substituted by halogen or lower alkoxy, and R' is lower alkyl. The term "lower alkyl" or "lower alkoxy" as used herein means an alkyl or alkoxy radical with 1 to 5 carbon atoms.

The compounds of formula I are valuable antibacterial agents, and are new compounds.

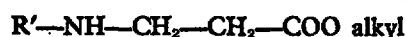
The process of the invention for the production of the aforesaid compounds comprises:

- a) condensing a 4-chloro-5-carbethoxy-pyrimidine of formula:

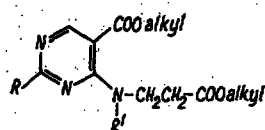


II

with a lower alkyl β-amino-propionate of formula:

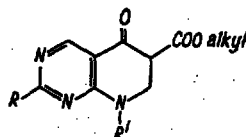


to form a 4-N-(β-carbalkoxyethylamino)-5-carbethoxy-pyrimidine of formula:



III

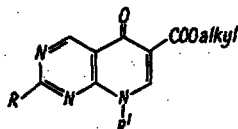
b) cyclising the compound of formula III by treatment with an alkali metal alcoholate to form a 5-oxo-6-carbalkoxy-5,6,7,8-tetrahydro-pyrido(2,3-d)pyrimidine of formula:



IV

c) halogenating the β -keto-ester of formula IV to yield the corresponding 6-halogeno derivative,

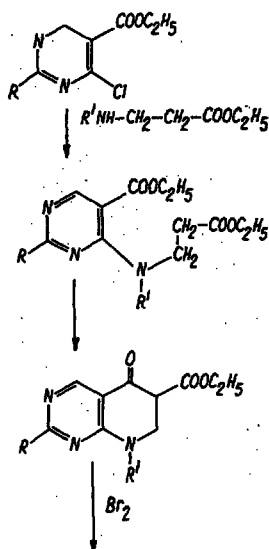
d) treating the said derivative with a base to bring about dehydrohalogenation to give a 6-carbalkoxy-5-oxo-5,8-dihydro-pyrido(2,3-d)pyrimidine of formula:



VI

and e) saponifying the compound of formula VI to yield the desired acid of formula I.

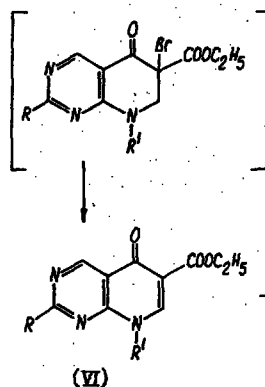
The starting material in this process is a 4-chloro-5-carbethoxy-pyrimidine (II), condensation of which with a lower alkyl (especially ethyl) β -aminopropionate, substituted at the nitrogen by the radical R' , leads to a 4-N-(β -carbalkoxyethylamino)-5-carbethoxy-pyrimidine (III). Cyclisation of the latter, under the effect of a (preferably tertiary) alkali metal alcoholate forms a 5-oxo-6-carbalkoxy-5,6,7,8-tetrahydropyrido(2,3-d)pyrimidine (IV). When this β -keto-ester is treated with a halogenating agent such as bromine or sulphuryl chloride, it yields a derivative halogenated in the 6-position (V) which is not isolated and which, when treated with a tertiary amine such as an aliphatic tertiary amine (for example triethylamine) or an aromatic tertiary amine (such as dimethylaniline) or a heterocyclic tertiary amine (such as pyridine and its methyl derivatives), undergoes dehydrohalogenation leading to a 6-carbalkoxy-5-oxo-5,8-dihydro-pyrido(2,3-d)pyrimidine (VI), saponification of which yields the desired acid (I). These reactions may be represented as follows:



(II)

(III)

(IV)



(V)

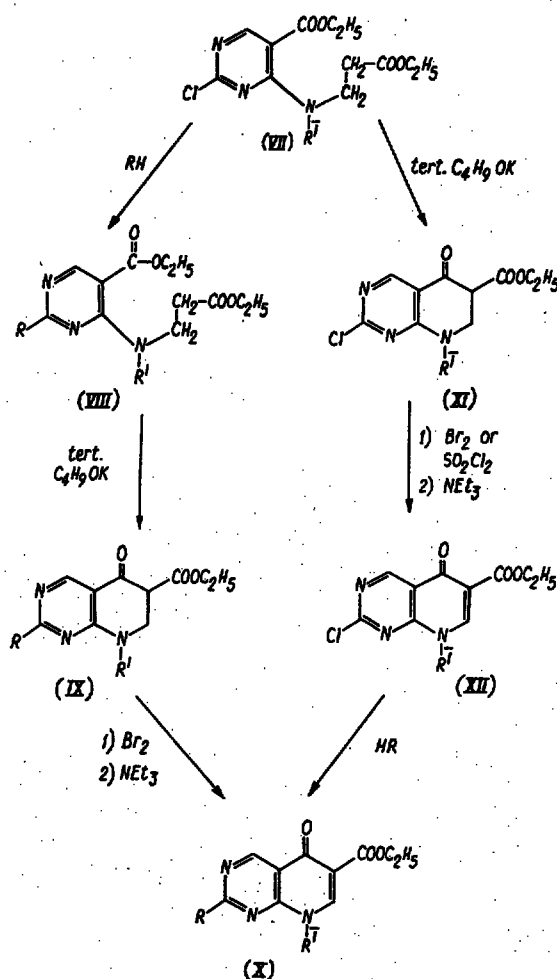
(I)

In a modification of this process, the starting material is 2,4-dichloro-5-carbethoxy-pyrimidine (II, R=Cl), condensation of which with an N-substituted ethyl β -amino-propionate gives 2-chloro-4- β -carbethoxyethylamino-5-carbethoxy-pyrimidine (VII), which can be used by one of two methods.

In the first method, it is treated under hot conditions with an amine of formula: HR, which leads to a N₂-substituted 2-amino-4- β -carbethoxyethylamino-5-carbethoxy-pyrimidine (VIII), which, when cyclised by an alkali metal alcoholate, preferably potassium tertiary butylate, yields the N₂-substituted 2-amino-5-oxo-6-carbethoxy-5,6,7,8-tetrahydropyrido(2,3-d)pyrimidine (IX) which, according to the methods indicated above, leads, after halogenation followed by dehydrohalogenation, to an ester carrying the amine group in the 2-position (X), saponification of which gives the corresponding acid.

In the second method, the 2-chloro-4- β -carbethoxyethylamino-5-carbethoxy-pyrimidine (VII) is cyclised by a tertiary alkali metal alcoholate, preferably potassium tertiary butylate to give a 2-chloro-5-oxo-6-carbethoxy-5,6,7,8-tetrahydro-pyrido(2,3-d)pyrimidine (XI), the halogenation followed by dehydrohalogenation of which yields a N₂-substituted 2-chloro-6-carbethoxy-5-oxo-5,8-dihydro-pyrido(2,3-d)pyrimidine (XII). The action of an amine of formula HR (generally at ordinary temperature) on the latter leads to an ester (X), saponification of which yields an acid according to the invention. The second method is particularly valuable when the radical R is sensitive to the halogenation, which leads to the formation of by-products during the stage (IX) $\rightarrow$ (X).

These reactions may be represented as follows:



The condensation of the 4-chloro-5-carbethoxy-pyrimidines (II) with the N-substituted ethyl β -aminopropionate is carried out at ambient temperature in a neutral solvent such as an aromatic hydrocarbon (benzene or toluene) or a halogenated hydrocarbon (for example chloroform or dichloroethane). Per mol of chloropyrimidine, either two mols of the amino-ester or one mol of the latter and one mol of a tertiary amine (e.g. triethylamine), used as an acceptor of the hydracid formed in the reaction, are employed. After standing for 2 to 10 hours, the solution is filtered, washed with water and dried. Evaporation leaves the ester (III) or (VII), in an approximately theoretical yield, most frequently as an oil which is difficult to crystallise and which can be used directly for the following operation.

One mol of ester (III) or (VII), preferably dissolved in an aromatic hydrocarbon (benzene or toluene), is added at ambient temperature to a solution of potassium tertiary butylate (1 mol), prepared by dissolving 1 gram atom of metal in anhydrous tertiary butanol. The potassium salt of the enolate of the β -keto-ester (IV) or (XI) precipitates from the start of the addition. After standing for at least two hours at ambient temperature, the mixture is taken up in iced water and the aqueous phase is separated by decanting and acidified with acetic acid (1 mol) to cause precipitation of the β -keto-ester which is extracted with a suitable solvent. The organic solution is dried and then evaporated *in vacuo*; the keto-ester (IV) or (XI) is purified by recrystallisation. The yield is generally between 75 and 80%.

For the halogenation, the ester (IV, IX or XI) is dissolved (at a concentration of, e.g., 10 to 20%) in a suitable solvent such as chloroform or acetic acid. The solution is stirred and cooled externally. One mol of bromine, as a 10% solution in chloroform or acetic acid, is added dropwise, taking care that the temperature remains below 15°C. After standing for 2 hours at ordinary temperature, the solvent is removed at a low temperature (40°C.) *in vacuo*. The residue is dissolved in 10 parts of chloroform, the solution is cooled, and a solution of 2.5 to 3 mols of a tertiary amine, preferably triethylamine, in the same solvent, is added to it at a temperature of between 10 and 15°C. After contact (two to ten hours), the solvent is evaporated *in vacuo*. The residue is taken up in water and the precipitate is filtered off, washed until the triethylamine hydrobromide has been completely removed, and recrystallised from a suitable solvent. The yields are generally between 75 and 80%. When the halogenation is carried out in chloroform, it is possible to add the tertiary amine directly, under the conditions described above, to the solution resulting from the addition of bromine to the β -keto-ester.

The halogenation can also be carried out with other reagents, especially sulphuryl chloride. The reaction is then preferably carried out in a chloroform solution. The addition of sulphuryl chloride to the solution of the β -keto-ester in equimolar proportions may be carried out at 15–20°C., and the reaction is completed at ambient temperature within a period of two to four hours. 2.5 to 3 mol proportions of triethylamine or other tertiary amine are then added to the solution which is kept at 10–15°C., and the dehydrohalogenation reaction is allowed to continue for 4 to 12 hours. The reaction product is isolated and purified as in the case where the halogenating agent is bromine.

The condensation of the 2-chloro-4-(N- β -carbethoxyethyl-N-alkyl)amino-5-carbethoxy-pyrimidine (VII) with the amine HR in a molar ratio of 1:2 is carried out at 70–100°C., in a neutral solvent with a suitable boiling point, e.g. benzene or toluene. Depending on the reactivity of the amine HR, the heating period can be from one to six hours. After cooling, the hydrochloride of the excess amine HR which has precipitated, is filtered off. Evaporation of the solvent gives the diester (VIII), either in solid form (in which case it is then purified by recrystallisation from a suitable solvent), or as an oil which cannot be crystallised and which is used directly for the following stages. The cyclisation with potassium tertiary butylate and the halogenation and dehydrohalogenation of the β -keto-ester (IX) are continued in accordance with the techniques described above.

The condensation of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido-(2,3-d)pyrimidine (XII) (1 mol) with the amine HR can be carried out either by using an excess of the latter, i.e. 2 to 2.5 molar proportions, as an acceptor for the hydracid formed in the reaction, or by employing 1 molar proportion of the amine HR and 1 to 1.5 molar proportions of a tertiary amine, (for example triethylamine), the latter playing the role of acid acceptor. The reaction can be carried out in an aromatic hydrocarbon (e.g. benzene or toluene), a halogenated aliphatic hydrocarbon (e.g. chloroform or dichloroethane) or a lower alcohol (e.g. ethanol or isopropanol).

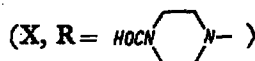
The amine HR, used in excess, or its mixture with the tertiary amine is added to the stirred solution or suspension (usually at 10 to 20% concentration) of the halo-

generated derivative. In the majority of cases, the reaction is rapid and complete at ordinary temperature. Where necessary, it is completed by heating at 80–100°C. for one to two hours. After evaporating the solvent *in vacuo*, the residue is taken up in water and the precipitate is filtered off and recrystallised from a suitable solvent.

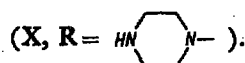
However, under these conditions, it is not practicable to obtain the esters (X) wherein R is a 1-piperazino group



by reacting piperazine with the chlorinated derivative (XII). In this case, no matter what the experimental conditions may be, the two imine groups of piperazine both react and a mixture of the mono-substituted and disubstituted derivatives is obtained. The desired mono-substituted compound can however be obtained by condensing a chlorinated derivative (XII) with 1-formyl-piperazine: a 2-(4'-formyl-piperazino) derivative



is thus obtained, which can then be deformylated, e.g. by treatment with a solution of hydrogen chloride in ethanol, to give the piperazino derivative



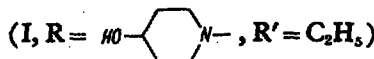
The esters (VI) and (X) may be saponified, most frequently in an aqueous-alcoholic medium, with an alkali metal hydroxide (1.2–1.5 molar proportions). At ordinary temperature, the reaction is generally complete in one to three hours. The excess ethanol is removed by evaporation *in vacuo* at a low temperature, the residue is dissolved in water, and the solution is rendered slightly acidic (pH 3 to 6). The acid (I) usually precipitates: it is then filtered off, washed and recrystallised from a suitable solvent. If, however, the acid (I) is soluble in water, the solution is saturated with a salt, e.g. by adding sodium chloride or sodium acetate, and the acid is extracted with a suitable solvent such as, for example, chloroform. After evaporating the latter, the acid is recrystallised from a suitable solvent.

Since certain compounds are sensitive to the action of strong bases, the saponification can also be carried out by heating the esters under reflux with a solution of an alkali metal carbonate. After cooling, the acids are isolated as above.

The following Examples illustrate the invention.

EXAMPLE I.

2-(4'-Hydroxy-piperidino)-5-oxo-8-ethyl-5,8-dihydropyrido(2,3-d)-pyrimidine-6-carboxylic acid



In a 1 litre 3-necked flask equipped with a mechanical stirrer, a dropping funnel and a thermometer, a solution of potassium tertiary butylate is prepared from 400 cm³ of the tertiary alcohol and 8 g. of potassium metal. This solution is brought to 20°C. and 66 g. of 2-chloro-4-β-carbethoxyethylamino-5-carbethoxypyrimidine are added to it and the mixture is stirred at ordinary temperature for 2 hours: the potassium derivative of the reaction product precipitates. The mixture is poured into 200 cm³ of iced water and the solution is brought to pH 3 by adding 4N hydrochloric acid with stirring. The precipitate is filtered off, washed with water and recrystallised from 320 cm³ of isopropanol and yields 46 g. (81.2%) of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,6,7,8-tetrahydro-pyrido(2,3-d)-pyrimidine; melting point 146°C.

Analysis for C₁₉H₁₄ClN₃O₃ (molecular weight 283.5)

Calculated %	C	50.79	H	5.07	N	14.81	Cl	12.50
Found %	C	51.10	H	5.14	N	14.61	Cl	12.68

64 g. (0.22 mol) of the above β -keto-ester are dissolved in 250 cm³ of chloroform. The solution is cooled in a bath of iced water and a solution of bromine (35.1 g., 0.44 gram atom) in 300 cm³ of chloroform is added to it at a rate such that the temperature of the reaction mixture remains between 10 and 15°C., which takes about 2 hours. The solution is stirred for a further hour at ambient temperature, the solvent is driven off *in vacuo* and the residue is taken up in 200 cm³ of anhydrous chloroform. The solution is stirred in a bath of iced water and a solution of triethylamine (50 g., 0.48 mol) in 100 cm³ of chloroform is added to it, dropwise, at a rate such that the temperature of the mixture remains between 15 to 10°C. (duration 2 hours).

When the addition is complete, the solution is stirred for a further hour at 20°C., the solvent is evaporated *in vacuo* and the residue is taken up in 200 cm³ of water. The insoluble matter is extracted with benzene and the organic solution is dried over MgSO₄.

Evaporation of the solvent leaves a residue which, after recrystallisation from 450 cm³ of a mixture of isopropyl ether (1 volume) and benzene (1 volume), yields 50 g. (78.1%) of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)-pyrimidine; melting point 158°C.

Analysis for C₁₂H₁₂ClN₃O₃ (molecular weight 281.5)

Calculated %	C 51.15	H 4.26	N 14.91	Cl 12.61
Found %	C 51.32	H 4.52	N 14.84	Cl 12.50

3.5 g. of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,6,7,8-tetrahydro-pyrido(2,3-d)-pyrimidine, dissolved in 15 cm³ of chloroform, are cooled and stirred at -5°C. 1.7 g. of sulphuryl chloride, dissolved in 10 cm³ of anhydrous chloroform, are added. The cooling mixture is removed and the solution is stirred at ordinary temperature for 2 hours. It is again cooled to 5-10°C., and a solution of 5.5 cm³ of triethylamine in 10 cm³ of chloroform is added to it, dropwise. The mixture is left to stand overnight at ambient temperature. The solvent is evaporated and the residue is taken up in 50 cm³ of water. The precipitate is filtered off, washed with water and dissolved in 50 cm³ of ethyl acetate. The solution is dried (MgSO₄), the solvent is evaporated and the residue is recrystallised from a mixture of isopropyl ether and benzene. 2.2 g. (63%) of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine are obtained; melting point 156°C. (identical to the product described above).

2.8 g. of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine and 2 g. of 4-hydroxy-piperidine, dissolved in 35 cm³ of toluene, are heated under reflux for 4 hours. After cooling, 20 cm³ of water are added to the mixture; the organic phase is washed again with water and dried over MgSO₄; the solvent is evaporated and the residue is recrystallised from ethanol and yields 1.75 g. of 2-(4'-hydroxy-piperidino)-5-oxo-6-carbethoxy-5,8-dihydro-pyrido(2,3-d)pyrimidine; melting point 205°C.

Analysis for C₁₇H₂₂N₄O₄ (molecular weight 346.38)

Calculated %	C 58.94	H 6.40	N 16.18
Found %	C 59.00	H 6.30	N 16.12

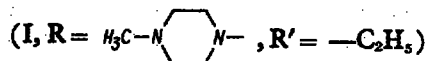
1.75 g. of this ester are added to a solution of sodium hydroxide (0.25 g.) in a mixture of alcohol (30 cm³) and water (2 cm³). The mixture is stirred for one hour at ambient temperature, the solvent is driven off *in vacuo* and the residue is dissolved in 10 cm³ of water. The solution is acidified by means of acetic acid. The precipitate is filtered off, washed with water and recrystallised from ethanol and yields 0.6 g. of 2-(4'-hydroxy-piperidino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)-pyrimidine-6-carboxylic acid. Melting point 244°C.

Analysis for C₁₅H₁₄N₄O₄ (molecular weight 318.33)

Calculated %	C 56.59	H 5.70	N 17.60
Found %	C 56.37	H 5.95	N 17.47

EXAMPLE II.

2-(4'-Methyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)-pyrimidine-6-carboxylic acid



8.4 g. (0.03 mol) of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine, suspended in 60 cm³ of ethanol, are stirred vigorously. A mixture of 3.3 g. (0.03 mol) of 1-methyl-piperazine and 3.3 g. of triethylamine, dissolved in 20 cm³ of ethanol, is added rapidly. The chlorinated derivative dissolves with evolution of heat. After stirring for 2 hours at ambient temperature, the solvent is driven off *in vacuo*, the residue is taken up in 50 cm³ of a saturated solution of Na₂CO₃ and the mixture is extracted with chloroform. After drying (Na₂SO₄) and evaporation, the residue (9.5 g.) is recrystallised from 100 cm³ of a mixture of isopropyl ether (1 volume) and benzene (1 volume) and yields 8.5 g. (85%) of 2-(4'-methyl-piperazino)-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine which melts in two stages: 146°C. and then 158°C.

Analysis for C₁₇H₂₃N₅O₃ (molecular weight 345.39)

Calculated % C 59.11 H 6.71 N 20.28

Found % C 59.23 H 6.68 N 20.42

25.3 g. of this ester (0.075 mol) are stirred with 100 cm³ of N NaOH solution. The solution is heated to 50—60°C., to dissolve the ester and then the saponification is completed at ambient temperature over the course of 2 hours. The solution is brought to pH 6 with acetic acid. After saturating the mixture with sodium acetate, the solution is extracted with 4 × 50 cm³ of chloroform.

The combined chloroform extracts are dried over MgSO₄, the solvent is evaporated and the residue is recrystallised from a mixture of ethanol (500 cm³) and dimethyl-formamide (100 cm³); 14.5 g. of acid are thus obtained. On concentrating the crystallisation mother liquors to $\frac{1}{3}$ of their volume, a further 1.5 g. of product are obtained, corresponding to a total yield of 16 g. (70%). 2-(4'-Methyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid melts 227°C.

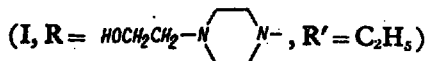
Analysis for C₁₈H₁₉N₅O₃ (molecular weight 317.34)

Calculated % C 56.77 H 6.04 N 22.07

Found % C 56.69 H 6.23 N 22.35

EXAMPLE III.

2-(4-β-Hydroxyethyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid



5.6 g. of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine, 5.2 g. of 1-β-hydroxyethyl piperazine and 50 cm³ of toluene are heated under reflux for 2 hours. After cooling, the solution is filtered to remove the hydrochloride which has precipitated, and the organic solution is diluted with its own volume of chloroform, is washed with water and dried (MgSO₄). After evaporation, the residue is recrystallised from 40 cm³ of a mixture of isopropanol (1 volume) and isopropyl ether (2 volumes). 5.14 g. (68%) of 2-(4-β-hydroxyethyl-piperazino)-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine are obtained; melting point 172°C.

Analysis for C₁₈H₂₅N₅O₄ (molecular weight 375.42)

Calculated % C 57.58 H 6.71 N 18.60

Found % C 57.53 H 6.57 N 18.80

5.14 g. of the ester are added to an aqueous-alcoholic solution of sodium hydroxide (N NaOH: 15 cm³, ethanol: 10 cm³); the mixture is stirred for 2 hours at ordinary temperature and is then brought to pH 6 by adding acetic acid. After concentration to dryness *in vacuo*, the residue is taken up in a saturated solution of sodium acetate (50 cm³) and is extracted with chloroform (3 × 50 cm³). After drying (MgSO₄), the solvent is evaporated and the residue is recrystallised from 80 cm³ of ethanol. 2.4 g.

(53.3%) of 2-(4'- β -hydroxyethyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid are obtained; melting point 222°C.

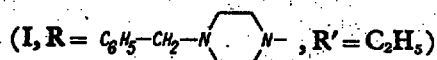
Analysis for $C_{16}H_{21}N_5O_4$ (molecular weight 347.37)

Calculated % C 55.32 H 6.09 N 20.16

Found % C 54.96 H 6.04 N 19.92

EXAMPLE IV.

2-(4'-Benzyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid



As described in Example III, a mixture of 5.6 g. of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine, 7.04 g. of N-benzylpiperazine and 80 cm³ of toluene is heated under reflux for 2 hours. After cooling, the mass is diluted with 100 cm³ of chloroform and is taken up in 200 cm³ of water. After washing the organic phase with water, the reaction product is isolated as described in the Example mentioned. It is recrystallised from isopropanol. 7.6 g (90%) of 2-(4'-benzyl-piperazino)-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine are obtained; melting point 152°C.

Analysis for $C_{23}H_{27}N_5O_3$ (molecular weight 421.49)

Calculated % C 65.54 H 6.46 N 16.62

Found % C 66.03 H 6.44 N 16.97

Saponification of 9.5 g. of this ester with an aqueous-alcoholic solution of sodium hydroxide (N NaOH, 15 cm³ + water, 30 cm³ + ethanol, 30 cm³) is complete in one hour at ordinary temperature. After acidification, the precipitate is filtered off and recrystallised from 160 cm³ of a mixture of dimethylformamide (1 volume) and ethanol (1 volume). 6.8 g. of 2-(4'-benzyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid are obtained; melting point 207°C.

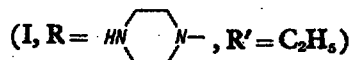
Analysis for $C_{21}H_{25}N_5O_3$ (molecular weight 293.43)

Calculated % C 64.11 H 5.89 N 17.80

Found % C 64.42 H 6.03 N 17.79

EXAMPLE V.

2-Piperazino-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)-pyrimidine-6-carboxylic acid



A mixture of 8.4 g of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine, 6.9 g of formylpiperazino and 160 cm³ of chloroform is heated under reflux for one hour. After cooling, the solution is washed with water and dried and the solvent is evaporated. The oily residue crystallises on being taken up in 100 cm³ of isopropyl ether. The solid is filtered off and recrystallised from 50 cm³ of ethanol and yields 8.9 g (82%) of 2-(4'-formylpiperazino)-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine which melts in two stages; melting point 195°C, solidification, followed by m.p. 204°C.

Analysis for $C_{17}H_{21}N_5O_4$ (molecular weight 359.38)

Calculated % C 56.81 H 5.89 N 19.49

Found % C 56.97 H 5.95 N 19.58

3.6 g of the above formyl derivative are suspended in 25 cm³ of a 5% strength solution of HCl in alcohol. After gentle heating (at 40—50°C) to facilitate dissolution, the mixture is left to stand for 8 hours at ordinary temperature. The dihydrochloride of the deformylated derivative precipitates. After being taken in 100 cm³ of ether, it is filtered off and suspended in 40 cm³ of water and the mixture, having been rendered alkaline by adding Na₂CO₃, is extracted with chloroform. After evaporating the solvent, the product is recrystallised from 40 cm³ of a mixture of isopropyl ether

(1 volume) and benzene (1 volume). 2.8 g (84%) of 2-piperazino-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine are obtained; melting point 155°C.

Analysis for $C_{16}H_{21}N_5O_3$ (molecular weight 331.37)

Calculated % C 57.99 H 6.39 N 21.14

Found % C 57.98 H 6.23 N 21.39

Saponification of this ester (3.3 g) with 30 cm³ of N/3 aqueous sodium hydroxide solution is complete in 2 hours at ordinary temperature. After acidification (pH about 6) with acetic acid, the precipitate is filtered off, washed with water and recrystallised from 60 cm³ of a mixture of dimethylformamide (1 volume) and ethanol (1 volume). 1.8 g of 2-piperazino-5-oxo-8-ethyl-5,8-dihydropyrido(2,3-d)pyrimidine-6-carboxylic acid are obtained; melting point 264°C.

Analysis for $C_{14}H_{17}N_5O_3$ (molecular weight 303.32)

Calculated % C 55.43 H 5.65 N 23.09

Found % C 55.68 H 5.67 N 23.25

EXAMPLE VI.

2-(4'-Phenyl-piperazino)-5-oxo-5,8-dihydro-pyrido(2,3-d)-pyrimidine-6-carboxylic acid



A solution of 3.2 g (0.02 mol) of N-phenyl-piperazine in 30 cm³ of toluene is added to 2.8 g (0.01 mol) of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine dissolved in 30 cm³ of toluene. Reaction takes place practically instantaneously with evolution of heat and the mixture sets solid due to formation of phenylpiperazine hydrochloride. The mixture is diluted with 100 cm³ of ethyl acetate, the solution is filtered, washed with water and dried (MgSO₄) and the solvent is evaporated. The residue is recrystallised from 110 cm³ of isopropanol and yields 3.38 g (84%) of 2-(4'-phenyl-piperazino)-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine; melting point 186°C.

Analysis for $C_{22}H_{25}N_5O_3$ (molecular weight 407.46)

Calculated % C 64.85 H 6.18 N 17.19

Found % C 64.97 H 6.08 N 16.74

When 2 g of this ester are saponified as indicated in Example V, they yield, after acidification and recrystallisation of the precipitate from 90 cm³ of a mixture of dimethylformamide (1 volume) and ethanol (1 volume), 1.4 g (77%) of 2-(4'-phenyl-piperazino)-5-oxo-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid; melting point 249°C.

Analysis for $C_{20}H_{21}N_5O_3$ (molecular weight 379.41)

Calculated % C 63.31 H 5.58 N 18.46

Found % C 63.49 H 5.71 N 18.36

EXAMPLE VII.

2-(4'-Methyl-piperazino)-5-oxo-8-methyl-5,8-dihydro-pyrido-(2,3-d)pyrimidine-6-carboxylic acid



26.2 g of ethyl N-methyl-β-aminopropionate, dissolved in 150 cm³ of benzene, are added dropwise to a solution, which is stirred and cooled to between 10 and 15°C; of 22 g of 2,4-dichloro-carbethoxypyrimidine in 150 cm³ of the same solvent. After standing overnight, the solvent is evaporated *in vacuo* and the residue is taken up in water (100 cm³) and extracted with ether. The organic solution is washed with water, dried (MgSO₄) and evaporated. The residue is recrystallised from 60 cm³ of hexane and yields 25 g (80%) of 2-chloro-4-(N-β-carbethoxyethyl-N-methyl)amino-5-carbethoxy-pyrimidine; melting point 60°C.

Analysis for $C_{13}H_{19}ClN_3O_4$ (molecular weight 315.5)

Calculated % C 49.45 H 5.74 N 13.31

Found % C 49.80 H 6.07 N 13.21

A solution of 10.5 g of this diester in 100 cm³ of benzene is added, with stirring and in the absence of moisture, to a solution of potassium tertiary butylate prepared from 1.4 g of potassium metal and 80 cm³ of tertiary butanol. The potassium derivative of the reaction product precipitates in the medium, which sets solid. After 2 hours at ordinary temperature, 2.5 cm³ of acetic acid followed by 300 cm³ of iced water are added, with stirring. The benzene phase is isolated by decanting, washed with water and dried (MgSO₄) and the solvent is evaporated. The residue is recrystallised from benzene and yields 5.7 g (63%) of 2-chloro-5-oxo-6-carbethoxy-8-methyl-5,6,7,8-tetrahydro-pyrido(2,3-d)pyrimidine; melting point 175°C.

Analysis for $C_{11}H_{12}ClN_3O_3$ (molecular weight 269.68)

Calculated % C 48.98 H 4.48 N 15.58 Cl 13.14

Found % C 49.00 H 4.70 N 15.34 Cl 13.10

A solution of 14 g of this β -keto-ester, dissolved in 100 cm³ of chloroform, is stirred and cooled to 10°C. A solution of 2.8 cm³ of bromine in 100 cm³ of chloroform is added to it, dropwise, at a rate such that the temperature of the reaction mixture remains between 10 and 15°C. The solution is left to stand at ambient temperature for one hour and is again cooled to 10°C, and a solution of triethylamine (16 cm³) in chloroform (100 cm³) is added to it dropwise.

After standing overnight at ordinary temperature, the solvent is removed *in vacuo* at 40°C. The residue is taken up in water (100 cm³), filtered off, washed to remove the triethylamine hydrobromide and dissolved again in chloroform (100 cm³); the solution is dried (MgSO₄), the solvent is evaporated and the residue is recrystallised from a mixture of isopropyl ether and benzene. 9.16 g (65.5%) of 2-chloro-5-oxo-6-carbethoxy-8-methyl-5,8-dihydro-pyrido(2,3-d)pyrimidine are obtained; melting point 205°C.

Analysis for $C_{11}H_{10}ClN_3O_3$ (molecular weight 267.67)

Calculated % C 49.34 H 3.76 N 15.69 Cl 13.24

Found % C 49.42 H 3.95 N 15.46 Cl 13.02

1.5 g of N-methylpiperazine are added to a stirred suspension of 2 g of 2-chloro-5-oxo-6-carbethoxy-8-methyl-5,8-dihydro-pyrido(2,3-d)pyrimidine in 20 cm³ of absolute ethanol. The reaction takes place with evolution of heat. After standing for 2 hours at room temperature, the solvent is evaporated *in vacuo*, the residue is taken up in water and the precipitate is filtered off, washed, dried *in vacuo* and recrystallised from benzene: 1.4 g (58%) of 2-(4'-methyl-piperazino)-5-oxo-6-carbethoxy-8-methyl-5,8-dihydro-pyrido(2,3-d)pyrimidine are obtained.

Analysis for $C_{16}H_{21}N_5O_3$ (molecular weight 331.37)

Calculated % C 57.99 H 6.39 N 21.14

Found % C 58.50 H 6.22 N 21.37

1.1 g of this ester are saponified by stirring for 2 hours at ordinary temperature in an aqueous-alcoholic solution of sodium hydroxide (NaOH 0.15 g, water 2 cm³, ethanol 5 cm³). The solvents are evaporated at 40°C *in vacuo*, the residue is taken up in 20 cm³ of water and the solution is acidified with acetic acid and then extracted with chloroform. Evaporation of the solvent leaves a residue which, after recrystallisation from a mixture of alcohol and dimethylformamide, yields 0.5 g of 2-(4'-methyl-piperazino)-5-oxo-8-methyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid; melting point 280°C.

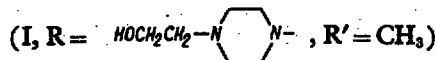
Analysis for $C_{14}H_{17}N_5O_3$ (molecular weight 303.32)

Calculated % C 55.43 H 5.65 N 23.09

Found % C 55.25 H 5.67 N 23.24

EXAMPLE VIII.

2-(4'-β-Hydroxyethyl-piperazino)-5-oxo-8-methyl-5,8-dihydropyrido(2,3-d)pyrimidine-6-carboxylic acid



5 As described in Example VII, reaction of N-β-hydroxyethyl-piperazine (2.1 g) with a suspension of 2 g of 2-chloro-5-oxo-6-carbethoxy-8-methyl-5,8-dihydro-pyrido(2,3-d)pyrimidine in 20 cm³ of ethanol (for 2 hours at ordinary temperature) yields, after evaporation of the solvent, a residue which is recrystallised from water, 2.14 g of 2-(4'-β-hydroxyethyl-piperazino)-5-oxo-6-carbethoxy-8-methyl-5,8-dihydro-pyrido(2,3-d)pyrimidine dihydrate are obtained; it has two melting points: melting point 160°C, solidification, followed by melting point 170°C.

Analysis for C₁₇H₂₃N₅O₄ · 2 H₂O (molecular weight 397.43)

Calculated % C 51.37 H 6.85 N 17.62

Found % C 51.60 H 6.53 N 18.01

15 1.94 g of this ester are saponified for 2 hours at ordinary temperature (NaOH 0.25 g, water 5 cm³, ethanol 10 cm³). After evaporating the solvent *in vacuo*, the residue is taken up in 20 cm³ of water and the solution is brought to pH 6 by adding acetic acid. The precipitate is filtered off, washed with water and recrystallised from a mixture of ethanol (1 volume) and dimethylformamide (2 volumes). 1.25 g of 2-(4'-β-hydroxyethyl-piperazino)-5-oxo-8-methyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid are obtained; melting point 245°C.

Analysis for C₁₈H₁₉N₅O₄ (molecular weight 333.34)

Calculated % C 54.04 H 5.75 N 21.01

Found % C 54.14 H 5.88 N 21.21

EXAMPLE IX.

2-(4'-Ethyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid



30 3.8 g of 2-chloro-5-oxo-6-carbethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine and 2.9 g of 1-ethyl-piperazine, dissolved in 50 cm³ of chloroform, are heated under reflux for 2 hours. After cooling, the organic solution is washed with water and dried over MgSO₄; the solvent is evaporated and the residue is recrystallised from a mixture of isopropyl ether (15 cm³) and benzene (25 cm³) and gives 4.4 g (yield: 93%) of 2-(4'-ethyl-piperazino)-5-oxo-8-ethyl-6-carbethoxy-5,8-dihydro-pyrido(2,3-d)pyrimidine; melting point 161°C.

Analysis for C₁₈H₂₅N₅O₄ (359.42)

Calculated % C 60.15 H 7.01 N 19.49

Found % C 60.22 H 6.81 N 19.66

40 Saponification of this ester (3.6 g) with a 2N solution of sodium hydroxide in alcohol, at ordinary temperature, yields, after the treatments described in the preceding examples, 2.23 g (67%) of 2-(4'-ethyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid; melting point 229°C, after recrystallisation from a mixture of ethanol (1 volume) and dimethylformamide (1 volume).

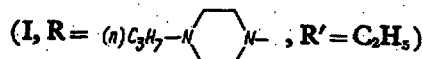
Analysis for C₁₈H₂₁N₅O₄ (molecular weight 331.37)

Calculated % C 57.99 H 6.39 N 21.14

Found % C 58.27 H 6.39 N 21.14

EXAMPLE X.

2-(4'-Propyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido-(2,3-d)pyrimidine-6-carboxylic acid



As described in Example IX, the condensation of 2.8 g of 2-chloro-5-oxo-6-carb-ethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine with 2.2 g of 1-propyl-piperazine, in chloroform (40 cm³) gives, after the treatments described in Example IX, 3.14 g of 2-(4'-propyl-piperazino)-5-oxo-6-carb-ethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine (yield 84%); melting point 149°C, after recrystallisation from ethyl acetate.

Analysis for C₁₇H₂₇N₅O₃ (molecular weight 373.45)

Calculated % C 61.10 H 7.29 N 18.75

Found % C 61.50 H 7.22 N 18.91

Saponification of this ester (2 g) as indicated in the preceding Examples, yields, after recrystallisation from a mixture of ethanol (1 volume) and dimethylformamide (2 volumes), 1.32 g (71%) of 2-(4'-propyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid; melting point 226°C.

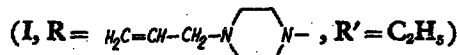
Analysis for C₁₇H₂₅N₅O₄ (molecular weight 345.39)

Calculated % C 59.11 H 6.71 N 20.28

Found % C 58.75 H 6.73 N 20.00

EXAMPLE XI.

2-(4'-Allyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido-(2,3-d)pyrimidine-6-carboxylic acid



2-(4'-Allyl-piperazino)-5-oxo-6-carb-ethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine is prepared, in a yield of 79%, by condensing 2-chloro-5-oxo-6-carb-ethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine with 1-allyl-piperazine, as indicated in the preceding Examples. It is a solid which melts in two stages; melting point 131°C, solidification, followed by melting point 143°C [after recrystallisation from a mixture of benzene (1 volume) and isopropyl ether (1 volume)].

Analysis for C₁₉H₂₅N₅O₃ (molecular weight 371.43)

Calculated % C 61.44 H 6.78 N 18.86

Found % C 61.10 H 6.75 N 18.75

Saponification of this ester with a 2 N solution of sodium hydroxide in alcohol gives 2-(4'-allyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid. When recrystallised from a mixture of ethanol (2 volumes) and dimethylformamide (1 volume), it is obtained in a yield of 64%; melting point 203°C.

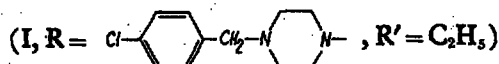
Analysis for C₁₉H₂₃N₅O₄ (molecular weight 343.38)

Calculated % C 59.46 H 6.16 N 20.40

Found % C 59.47 H 5.98 N 20.38

EXAMPLE XII.

2-(4'-p-Chlorobenzyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid



Condensation of 1-p-chlorobenzyl-piperazine with 2-chloro-5-oxo-6-carb-ethoxy-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine, as described in Example IV, gives, in a yield of 74%, 2-(4'-p-chlorobenzyl-piperazino)-5-oxo-

6 - carbethoxy - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine; melting point 150°C, after recrystallisation from ethyl acetate.

Analysis for $C_{23}H_{26}ClN_5O_3$ (molecular weight 455.5)

Calculated % C 60.59 H 5.70 N 15.36 Cl 7.79

Found % C 60.29 H 5.72 N 15.38 Cl 7.76

Saponification of this ester gives the corresponding acid; melting point 216°C, when purified by recrystallisation from dimethylformamide (yield 68%).

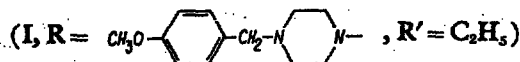
Analysis for $C_{21}H_{22}ClN_5O_3$ (molecular weight 427.5)

Calculated % C 58.94 H 5.14 N 16.37 Cl 8.30

Found % C 58.88 H 5.15 N 16.42 Cl 8.40

EXAMPLE XIII.

2-(4'-p-Methoxybenzyl-piperazino)-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid



By condensing 2 - chloro - 5 - oxo - 6 - carbethoxy - 8 - ethyl - 5,8 - dihydro-pyrido(2,3-d)pyrimidine with 1-p-methoxybenzylpiperazine, following the procedure described in Example IV, 2 - (p - methoxybenzyl - piperazino) - 5 - oxo - 6 - carbethoxy - 8 - ethyl - 5,8 - dihydro-pyrido(2,3 - d)pyrimidine is obtained and is purified by recrystallisation from a mixture of isopropyl ether (1 volume) and benzene (1 volume); melting point 136°C; yield 70%.

Analysis for $C_{24}H_{29}N_5O_4$ (molecular weight 451.51)

Calculated % C 63.84 H 6.47 N 15.51

Found % C 63.75 H 6.41 N 15.31

Saponification gives the corresponding acid; melting point 200°C when purified by recrystallisation from dimethylformamide; yield 60%.

Analysis for $C_{22}H_{25}N_5O_4$ (molecular weight 423.46)

Calculated % C 62.40 H 5.95 N 16.55

Found % C 62.42 H 5.93 N 16.55

The anti-microbial activity of the compounds of the invention has been demonstrated *in vitro* on two different nutrient media:

a) Trypticase soya medium (T.S.A.), which is very suitable for culturing the majority of microorganisms; it has the following composition:

Trypsin-produced peptone from casein	15 g
Papain-produced peptone from soya	5 g
Sodium chloride	5 g
Agar	15 g
Water, q.s.p.	1,000 cm ³

After sterilisation, the pH is 7.3.

b) Ordinary nutrient agar (N.A.), which is less rich in nutrients, corresponds to the following formulation:

Meat extract	3 g
Peptone	5 g
Agar	15 g
Water, q.s.p.	1,000 cm ³

After sterilisation at 120°C, the pH is 6.8.

Table I gives the minimum inhibitory concentrations (expressed in $\mu\text{g}/\text{cm}^3$) for the various microorganisms tested on each medium.

The products were tested on three Gram positive microorganisms (1 to 3) and on 10 Gram negative microorganisms (4 to 13).

The minimum inhibitory concentrations (M.I.C.) were determined by dilution

in the agar media, the ranges of concentration extending from 0.2 to 100 $\mu\text{g}/\text{cm}^3$, as a geometric progression with a ratio of 2. Inoculation of the dishes was carried out by means of a multiple inoculator with 10^{-3} dilutions of 18 hour broth cultures. The dishes are placed in an oven at 37°C and readings are made after 18 hours' incubation. The M.I.C. is the lowest concentration which completely inhibits the culture.

The compounds of Examples I, II, III, IV, V, VII and VIII to XIII are particularly active against Gram-negative microorganisms, the minimum inhibitory concentration being generally substantially lower in the ordinary medium (N.A.) than in the trypticase-soya medium (T.S.A.).

The compounds of the invention can be used in human or veterinary therapy, especially as anti-bacterial agents, in the form of a pharmaceutical composition in which they are combined with a non-toxic pharmaceutical carrier which is preferably such that the composition can be administered orally or parenterally. The oral form preferably consists of tablets or gelatine-coated pills each containing 50 to 500 mg. of active principle, preferably 2-piperazino-5-oxo-8-ethyl-5,8-dihydro-pyrido[2,3-d]-pyrimidine-6-carboxylic acid.

For the parenteral forms, it is possible to use aqueous solutions containing 5 to 10% of active product, and having a pH between 6 and 7; isotonicity is achieved by adding sodium chloride if necessary. With compounds which are less soluble in water than the limits fixed above, aqueous solutions of polyethylene glycol (300 or 600) or propylene glycol, at concentration 10 to 40%, may be used as the solvent. Tertiary amides of lower aliphatic acids such as N-dimethylacetamide and N-diethylacetamide and lactamide, or 5% strength aqueous solutions of benzyl alcohol can also be used as solubilisation adjuvants. The solutions thus produced can be sterilised either by sterile filtration or by autoclaving. They may be dispensed into 5 or 10 cm^3 ampoules.

TABLE 1

Microorganism	Ex. I		Ex. II		Ex. III		Ex. IV		Ex. V		Ex. VII		Ex. VIII	
	T.S.A.	N.A.	T.S.A.	N.A.	T.S.A.	N.A.	T.S.A.	N.A.	T.S.A.	N.A.	T.S.A.	N.A.	T.S.A.	N.A.
1 Staphylococcus 209 P	50	1.6	100	3.1	12.5	3.1	3.1	0.8	12.5	12.5	100	50	>100	25
2 Streptococcus Group A	12.5	12.5	6.2	25	12.5	3.1	3.1	0.8	25	25	100	50	>100	25
3 B. subtilis	3.1	0.8	6.2	1.6	3.1	1.6	0.8	0.4	6.2	3.1	>100	100	100	12.5
4 Bord. bronchiseptica	>100	100	100	50	>100	50	>100	3.1	50	12.5	100	25	100	12.5
5 B. pyocyaneus A22	>100	100	100	12.5	>100	50	>100	100	12.5	25	>100	50	>100	100
6 B. pyocyaneus TUR	>100	100	100	50	>100	50	>100	100	>100	25	>100	50	>100	100
7 Esch. Coli 95	12.5	6.2	6.2	0.8	3.1	1.6	12.5	1.6	0.8	0.8	6.2	1.6	25	1.6
8 Klebs. pneumoniae	0.8	0.4	6.2	0.4	1.6	0.4	1.6	0.4	1.6	0.8	6.2	6.2	12.5	3.1
9 Salm. typhi	12.5	6.2	3.1	3.1	6.2	12.5	12.5	1.6	1.6	6.2	12.5	3.1	50	6.2
10 Sh. sonnei	12.5	3.1	1.6	0.8	1.6	1.6	12.5	1.6	0.8	0.8	3.1	12.5	12.5	1.6
11 Pr. vulgaris	12.5	3.1	6.2	1.6	6.2	50	50	12.5	1.6	6.2	25	3.1	50	25
12 Pr. mirabilis	25	12.5	6.2	6.2	12.5	3.1	100	6.2	0.8	0.8	12.5	3.1	25	6.2
13 Pr. morganii	0.8	0.4	1.6	0.8	1.6	1.6	12.5	3.1	0.8	0.8	6.2	3.1	25	3.1

Table (II) gives the minimum inhibitory concentrations of the compounds of Examples IX to XIII, determined on the agar medium, N.A., and expressed in $\mu\text{g}/\text{cm}^3$.

TABLE II

Microorganism	Ex. IX	Ex. X	Ex. XI	Ex. XII	Ex. XIII
1) Staphylococcus 20 P	1.6	6.2	3.1	0.8	0.4
2) Streptococcus Group A	3.1	6.2	1.6	1.6	0.8
3) B. subtilis	100	3.1	3.1	1.6	50
4) Bord. bronchiseptica	100	12.5	3.1	3.1	12.5
5) B. pyocyaneus A22	100	100	25	50	12.5
6) Esch. coli 95	0.8	50	50	100	3.1
7) Klebs. pneumoniae	0.4	1.6	0.4	0.8	0.2
8) Salm. typhi	0.8	12.5	3.1	12.5	1.6
9) Sh. sonnei	0.8	1.6	0.8	1.6	0.8
10) Pr. vulgaris	0.8	6.2	3.1	12.5	6.2
11) Pr. mirabilis	6.2	12.5	6.2	12.5	12.5
12) Pr. morganii	0.8	6.2	1.6	6.2	1.6

The compound of Example V appears to be the most valuable for therapeutic use. It has been tested on 25 bacterial strains, in comparison with nalidixic acid and 2 - pyrrolidino - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6-carboxylic acid.

The minimum inhibitory concentrations were determined five times on each strain by the solid medium (nutrient agar) dilution method. Table III gives the average values found for the three compounds.

It is apparent from this table that the compound of Example V is more active on the majority of the Gram negative microorganisms than the 2 other substances.

Furthermore, this product is characterised by a very low toxicity; when administered orally at doses as high as 4 g per kilogram, no deaths were observed amongst the rodents (rats and mice) and a dog was able to undergo a 6 weeks' treatment at 100 mg/kg/day without showing any toxic signs.

When administered orally, the compound of Example V is absorbed rapidly and is eliminated principally in the urine. Even with low doses, urinary concentrations are obtained which are very much greater than the minimum inhibitory concentrations effective against the majority of Gram negative microorganisms.

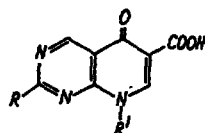
In an experiment involving a 20 kg dog, 50 mg of the compound of Example V, corresponding to 2.5 mg/kg, were administered by means of a probang. The urine of the animal was removed by catheterisation at the 3rd and at the 7th hour, and the anti-bacterial activity of these samples was measured, taking the compound administered as the reference product. Respective concentrations of 185 $\mu\text{g}/\text{cm}^3$ (3 hours) and 90 $\mu\text{g}/\text{cm}^3$ (7 hours) were thus found, the latter concentration being still very much greater than the minimum inhibitory concentration for the majority of the Gram negative microorganism investigated, especially the Colibacilli and Proteus.

TABLE III (Continued)

Microorganism	Nalidixic acid		2-Pyrrolidino-5-oxo-8-ethyl-5,8-dihydro-pyrido[2,3-d]pyrimidine-6-carboxylic acid		Ex. V	
	average	standard deviation	average	standard deviation	average	standard deviation
Pr. vulgaris 12-53	0.88	0.438	5.6	4.08	0.36	0.09
Pr. mirabilis Nig	3.42	1.68	30	11.18	1.58	0.3
Pr. morganii A 236	0.36	0.26	0.56	0.22	0.4	0

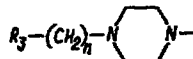
WHAT WE CLAIM IS:—

1. Process for the preparation of an 8-alkyl-5-oxo-5,8-dihydro-pyrido(2,3-d)-pyrimidine-6-carboxylic acid of the formula:



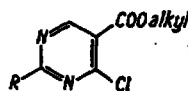
(I)

in which R is 4-hydroxy-piperidino, piperazino, or N-substituted piperazino of the formula:



in which n is 0, 1, 2 or 3 and R₃ is hydrogen, hydroxyl, vinyl, phenyl or phenyl substituted by halogen or lower alkoxy, and R' is lower alkyl, which comprises:

- a) condensing a 4-chloro-5-carbethoxy-pyrimidine of formula

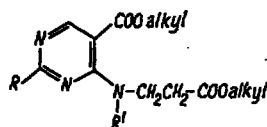


(II)

with a lower alkyl-β-amino-propionate of formula:



to form a 4-N-(β-carbalkoxyethylamino)-5-carbethoxy-pyrimidine of formula:



(III)

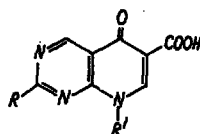
b) cyclising the compound of formula III by treatment with an alkali metal alcoholate to form a 5-oxo-6-carbalkoxy-5,6,7,8-tetrahydro-pyrido(2,3-d)pyrimidine of formula:

TABLE III (Continued)

Microorganism	Nalidixic acid		2-Pyrrolidino-5-oxo-8-ethyl-5,8-dihydro-pyrido-[2,3-d]pyrimidine-6-carboxylic acid		Ex. V	
	average	standard deviation	average	standard deviation	average	standard deviation
Pr. vulgaris 12-53	0.88	0.438	5.6	4.08	0.36	0.09
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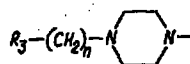
WHAT WE CLAIM IS:—

1. Process for the preparation of an 8-alkyl-5-oxo-5,8-dihydro-pyrido(2,3-d)-pyrimidine-6-carboxylic acid of the formula:



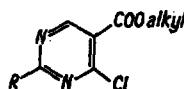
(I)

in which R is 4-hydroxy-piperidino, piperazino, or N-substituted piperazino of the formula:



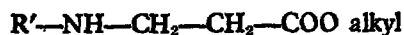
in which n is 0, 1, 2 or 3 and R₃ is hydrogen, hydroxyl, vinyl, phenyl or phenyl substituted by halogen or lower alkoxy, and R' is lower alkyl, which comprises:

a) condensing a 4-chloro-5-carbethoxy-pyrimidine of formula

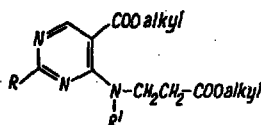


(II)

with a lower alkyl-β-amino-propionate of formula:



to form a 4-N-(β-carbalkoxyethylamino)-5-carbethoxy-pyrimidine of formula:



(III)

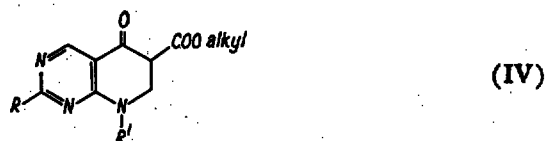
b) cyclising the compound of formula III by treatment with an alkali metal alcoholate to form a 5-oxo-6-carbalkoxy-5,6,7,8-tetrahydro-pyrido(2,3-d)pyrimidine of formula:





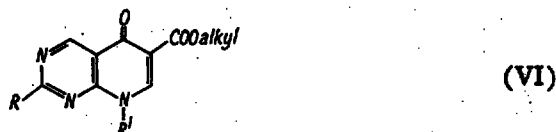
TABLE III

Microorganism	Nalidixic acid		2-Pyrrolidino-5-oxo-8-ethyl-5,8-dihydro-pyrido-[2,3-d]pyrimidine-6 carboxylic acid		Ex. V	
	average	standard deviation	average	standard deviation	average	standard deviation
<i>Staphylococcus</i> 209P	16.24	8.4	3.42	1.68	6.22	3.5
„ 9144	25	8.84	4.04	2.06	6.22	3.5
„ SIM	56.25	31.45	14.98	8.42	12.48	3.33
<i>Streptococcus</i> A561	18.74	19.65	2.82	2.1	13.74	6.86
„ DM19	100	0	100	0	100	0
<i>B. subtilis</i> 6633	72	0.75	1	0.6	8.1	9.54
<i>Bord. bronch.</i> 4617	25	17.67	80	27.36	75	35.35
<i>Ps. aeruginosa</i> A ₂₂	60	37.91	100	0	12.5	0
„ 72-345	60	37.91	75	35.35	13.74	6.86
<i>Esch. coli.</i> 95 I.S.M.	21.87	6.25	32.5	16.77	5.14	4.57
„ 54-127OMS	2.15	1.14	15.625	9.5	5.66	5
„ LRB 45	3.1	2.16	15.625	9.5	2.04	1.019
„ LRB 67	2.7	0.75	12.5	0	1.2	0.565
„ Cho	4.04	2.41	32.5	16.77	2.66	2.15
„ 111 B4	2.34	1.14	15.62	9.38	1.64	1.34
<i>Klebs. pneumoniae</i> 10.031	1.4	0.4	1.2	0.33	1.88	1.039
<i>Salm. typhi</i> 0901	2.7	0.75	23.74	16.77	2.06	2.063
<i>S. enteritidis</i> DANYZ	2.75	2.3	21.9	18.75	1.2	0.565
<i>S. orienburg</i> 10-66	100	0	100	0	27.5	7.5
Arizona 6211	3.12	1.7	27.5	13.7	1.26	1.138
<i>Providencia</i> 0223	100	0	100	0	16.24	8.4
<i>Sh. sonnei</i> I.P.S.	1.58	0.77	10.6	8.74	0.56	0.22



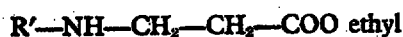
c) halogenating the β -keto-ester of formula IV to yield the corresponding 6-halogeno derivative,

d) treating the said derivative with a base to bring about dehydrohalogenation to give a 6-carbalkoxy-5-oxo-5,8-dihydro-pyrido(2,3-d)pyrimidine of formula:

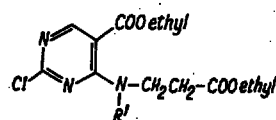


and e) saponifying the compound of formula VI to yield the desired acid of formula I.

2. Modification of the process according to claim 1, in which, in step a), a 2,4-dichloro-5-carbethoxy-pyrimidine is condensed with the ethyl β -aminopropionate

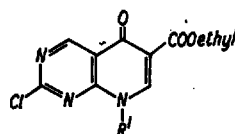


to form a 2-chloro-4- β -carbethoxyethylamino-5-carbethoxy-pyrimidine of formula:

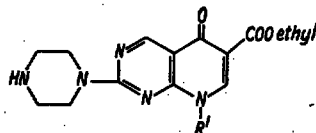


and, either between step (a) and step (b) or between step (d) and step (e), the 2-position chlorine atom is reacted with an amine of formula HR to introduce a radical —R in the 2-position.

3. Process according to claim 2, in which, between step (d) and step (e) the 2-chloro-6-carbethoxy-5-oxo-5,8-dihydro-pyrido(2,3-d)pyrimidine



is condensed with 1-formyl-piperazine to obtain a 2-(4-formyl-piperazino) derivative which is deformylated to give the piperazino derivative of formula



which is then saponified in step (e).

4. Process according to claim 3 in which the deformylation is effected with hydrogen chloride in ethanol.

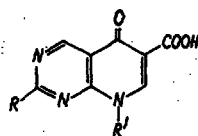
5. Process according to any of claims 1 to 4, in which in step (c) the halogenation is effected with bromine or sulphur chloride.

6. Process according to any of claims 1 to 5 in which in step (d) the dehydrohalogenation is effected with a tertiary amine.

7. Process according to claim 1 or 2 substantially as described in any one of the foregoing Examples I to XIII.

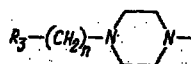
8. A compound of formula I when produced by the process of any of claims 1 to 7.

9. 8-Alkyl-5-oxo-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid of the formula:



I

in which R is 4-hydroxy-piperidino, piperazino or an N-substituted piperazino of formula:



in which n is 0, 1, 2 or 3, and R₃ is hydrogen, hydroxyl, vinyl, phenyl, or phenyl substituted by halogen or lower alkoxy, and R' is lower alkyl.

10. 2 - (4 - Methyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3-d)pyrimidine-6-carboxylic acid.

11. 2 - Piperazino - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine-6-carboxylic acid.

12. 2 - (4 - Hydroxy - piperidino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3-d)pyrimidine-6-carboxylic acid.

13. 2 - (4' - β - Hydroxyethyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro-pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

14. 2 - (4' - Benzyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

15. 2 - (4' - Methyl - piperazino) - 5 - oxo - 8 - methyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

16. 2 - (4' - β - Hydroxyethyl - piperazino) - 5 - oxo - 8 - methyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

17. 2 - (4' - Ethyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

18. 2 - (4' - Propyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

19. 2 - (4' - Allyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

20. 2 - (4' - *p* - Chlorobenzyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro-pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

21. 2 - (4' - *p* - Methoxybenzyl - piperazino) - 5 - oxo - 8 - ethyl - 5,8 - dihydro - pyrido(2,3 - d)pyrimidine - 6 - carboxylic acid.

22. A compound as claimed in any of claims 9 to 21 when produced by the process of any of claims 1 to 7.

23. A pharmaceutical composition containing, as active ingredient, a compound as claimed in any of claims 9 to 22 combined with a non-toxic pharmaceutical carrier.

24. A composition according to claim 23 containing, as active ingredient, 2-piperazino-5-oxo-8-ethyl-5,8-dihydro-pyrido(2,3-d)pyrimidine-6-carboxylic acid, and in the form of a tablet or gelatine-coated pill containing 50—500 mg of active principle.

25. A composition according to claim 23 substantially as hereinbefore described.

J. A. KEMP & CO.,
Chartered Patent Agents,
14, South Square,
Gray's Inn, London, W.C.1.

Reference has been directed in pursuance of Section 9, Subsection (1) of the Patents Act 1949, to Patent No. 1,129,358.