

PATENT SPECIFICATION

(11) 1 580 998

1 580 998

(21) Application No. 25379/78 (22) Filed 31 May 1978

(31) Convention Application No. 5118/77

(32) Filed 2 Aug. 1977 in

(33) Czechoslovakia (CS)

(44) Complete Specification published 10 Dec. 1980

(51) INT CL³ C07D 241/52 A61K 31/495

(52) Index at acceptance

C2C 1628 213 247 250 252 25Y 30Y 326 327 334 351 354 376 625
62Y 656 802 80Y AA MD

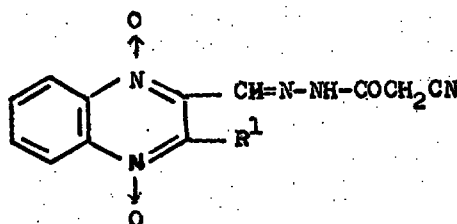


(54) 2-FORMYLQUINOXALINE-1,4-DIOXIDE CYANOACETYLHYDRAZONES, PROCESSES FOR THE PREPARATION THEREOF AND COMPOSITIONS CONTAINING SAME

(71) We, SPOFA, SPOJENE PODNIKY PRO
ZDRAVOTNICKOU VYROBU, a Czechoslovakian Corporation of Prague,
Czechoslovakia, 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:—

This invention relates to novel cyanoacetylhydrazones having antimicrobial
action.

According to the present invention there is provided a 2 - formylquinoxaline -
1,4 - dioxide cyanoacetylhydrazone of the general formula I



in which R¹ is a hydrogen atom or an alkyl group having 1 to 4 carbon atoms.

Certain acylhydrazones of the parent 2-formylquinoxaline-1,4-dioxide and its
derivatives are already known (United States Patent No. 3,433,871).

Surprisingly it has now been found that the cyanoacetylhydrazones of 2 -
formylquinoxaline - 1,4 - dioxide of formula I, which are hitherto unreported
substances, possess—in addition to valuable antimicrobial action both on Gram-
positive and Gram-negative microorganisms—also markedly pronounced
anticoccidial properties, promote growth in farm animals, and favourably
influence their general health.

In comparison with the known non-antibiotic animal growth promoters
routinely employed in practice, the compounds of the invention have significant
advantage in their substantially diminished toxicity. Thus, a comparative study of
the toxic properties of the 2 - formylquinoxaline - 1,4 - dioxide
cyanoacetylhydrazone of the invention (compound A) and, as a reference
substance, the known 2-formylquinoxaline-1,4-dioxide
methoxycarbonylhydrazone (compound B) was made in a series of parallel
experiments performed on male rats of 190 to 240 g body weight grouped in groups
of 10 animals each. The compounds under test were administered orally in the form
of 2.5% suspension in an aqueous gum arabic solution. Single doses as high as 500
mg/kg (2 ml. of a suspension) were given to the animals twice a day, also 1 g/kg
daily, always at 09.00 and 13.00 hours over five successive days, i.e., altogether ten
doses of either compound were administered. Table I below reports the mortality
rate of the individual animals upon administering the respective overall dose of the
compound under study.

(13)

2

1,580,998

2

TABLE I

Mortality of Test Animals Following the Indicated Dosage

	Control Group Rat No.	Reference/ compound B Overall dose, g/kg	Test Group Rat No.	Compound A Overall dose, g/kg	
5	1	2.5	1	—	
	2	1.5	2	—	
	3	2.0	3	—	
10	4	5.0	4	—	10
	5	2.0	5	—	
	6	2.0	6	—	
	7	3.0	7	—	
	8	3.0	8	4.5	
15	9	4.0	9	—	15
	10	3.0	10	5.0	

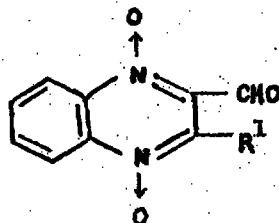
As evident from the above Table, the compound A of the invention is significantly less toxic than the reference compound B: all the animals of the control group died whereas, only two perished out of the ten in the test group treated with the compound of the invention. (The animals were observed for an additional 5 days following the day of administration of the last dose). An attempt was made to determine, using standard testing techniques, the LD_{50} value of compound A on female Wistar rats of 140 to 160 g body weight, grouped again in groups of ten animals each. The tested compound A was administered orally, in a single dose, in the form of a 20% suspension in Dorfman medium. This attempt to estimate the acute toxicity, however, failed since it was impossible to increase the administered doses of the tested compound over 5,500 mg/kg, which threshold dose was still sublethal. Consequently, for compound A, LD_{50} is substantially higher than 5,500 mg/kg orally.

As demonstrated by a series of experiments on broiler chickens, piglets and hogs, the compounds of the invention markedly stimulate the total performance of farm animals, particularly by increasing their liveweight gains, enhancing the utilization of feed mixtures, reducing the incidence of diarrhoea in piglets, and thus contributing to improvement of their general health. Also, the compounds of the invention can advantageously be employed, either individually or if required in combination with known veterinary medicines, for the prevention and treatment of salmonellosis and dysenteries in hogs and other farm animals.

The compounds can be administered either *per se* in pure state or preferably as mixtures with common carriers and/or excipients, in dispensing forms acceptable for veterinary purposes and animal nutrition, most preferable as concentrates and premixes suitable for the compounding of feed mixtures.

Several methods are available for the preparation of the compounds of the invention.

The compounds of the invention, of the general formula I, can be prepared by a method comprising reacting a 2 - formylquinoxaline - 1,4 - dioxide of the general formula II



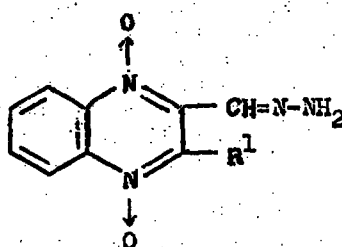
in which R^1 is as defined above, or an acetal thereof, with cyanoacetylhydrazine of the formula



This reaction is conveniently carried out in an inert organic or inorganic solvent or diluent or a mixture thereof; for example aliphatic or aromatic

hydrocarbons or their halogenated derivatives, e.g., benzene, toluene, chlorobenzene, dichloromethane or chloroform, carboxylic acids such as, e.g. formic or acetic acid, or aliphatic alcohols, e.g. methanol or ethanol, if required with addition of an organic or inorganic acid such as, e.g., formic, acetic, hydrochloric or p-toluene-sulphonic acid or mixtures thereof. The reaction proceeds with a sufficient reaction rate even at ambient temperature and can be conducted either at lowered or elevated temperatures, suitably ranging from approximately 0°C to the boiling temperature of the reaction mixture. The reaction time required is dependent on the nature of the starting materials used as well as on the reaction medium and temperature chosen, and generally varies from several minutes to approximately 24 hours. The reaction yields are as a rule nearly quantitative.

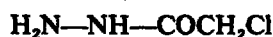
Another convenient process for the production of the compounds of the invention comprises reacting a 2 - formylquinoxaline - 1,4 - dioxide hydrazone of the general formula III



in which R¹ is as defined above is reacted with a reactive derivative of cyanoacetic acid, preferably with cyanoacetyl chloride or a cyanoacetic acid ester, particularly a halogenated phenyl cyanoacetate, in an inert organic or inorganic solvent or diluent or in a mixture thereof at a temperature of from -20°C to +100°C.

The starting parent 2 - formylquinoxaline - 1,4 - dioxide with hydrazine. Said reactive derivatives of cyanoacetic acid are known from the literature or can also be prepared by known methods.

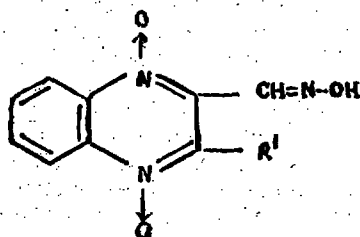
Still another process for the production of the compounds of the invention comprises reacting a 2 - formylquinoxaline - 1,4 - dioxide of the general formula II defined above with chloroacetylhydrazine of the formula.



in an inert organic or inorganic solvent or diluent or in a mixture thereof and reacting the product with an alkali metal cyanide.

A further process for the production of the compounds of the invention comprises reacting a 2-formylquinoxaline-1, 4-dioxide hydrazone of the general formula III defined above successively with chloroacetyl chloride in the presence of an organic or inorganic base, preferably an alkali metal carbonate or hydrogen carbonate or a tertiary amine, which is conveniently pyridine or triethylamine, in an inert organic or inorganic solvent or diluent or in a mixture thereof, and thereafter with an alkali metal cyanide.

Still alternatively, it is also possible to prepare the compounds of the invention by reacting a 2-formylquinoxaline-1, 4-dioxide oxime of the general formula IV



in which R¹ is as defined above with cyanoacetylhydrazine in an aqueous inorganic acid solution, preferably in 30 to 98 weight percent sulphuric acid, at a temperature from 20 to 100°C.

(2)

The following examples illustrate the invention. The melting points quoted in the Examples were determined on a Kofler block and are uncorrected.

Example 1

2-Formylquinoxaline-1,4-dioxide Cyanoacetylhydrazone

Method A

A solution of 15.2 g (0.08 mole) of 2-formylquinoxaline-1, 4-dioxide in 150 ml of ethanol was treated with 7.9 g (0.08 mole) of cyanoacetylhydrazine (melting at 105—107°C). The mixture was stirred for 4 hours at room temperature and the precipitated crude product was filtered off under suction. The yield was 18.4 g (85% of theory) of the crude compound. Upon crystallization from dimethylformamide, yellow crystals melting at 250—260°C (with decomposition) were obtained.

Method B

A mixture of 23.6 g (0.1 mole) of 2-formylquinoxaline-1, 4-dioxide dimethylacetal and 500 ml of methanol was treated with 200 ml of concentrated hydrochloric acid and agitated until complete dissolution. Then 10.5 g (0.105 mole) of cyanoacetylhydrazine were added and the reaction mixture was stirred 3 hours at room temperature. The precipitated crude product was filtered off under suction, washed with methanol and dried. The yield was 24.4 g (90% of theory) of a yellow substance melting at 255—260°C (decomposition).

Example 2

2-Formyl-3-methylquinoxaline-1,4 Dioxide Cyanoacetylhydrazone

A suspension of 20.4 g (0.1 mole) of 2 - formyl - 3 - methylquinoxaline - 1,4 - dioxide (m.p. 180—182°C) and 9.9 g (0.1 mole) of cyanoacetylhydrazine in 200 ml of ethanol was stirred for 4 hours at room temperature, and the separated product was filtered off under suction and washed with ethanol. The yield was 22.2 g (78% of theory) of the crude substance. Upon crystallization from diethylformamide, yellow crystals melting at 255—260°C (decomposition) were obtained.

Example 3

A commercial standard complete feed mixture (BR-1) for feeding broiler chickens was enriched by admixing to it, in a quantity of 1% by wt., a feed supplement containing active ingredients in the following ratio:

2-Formylquinoxaline-1,4-dioxide cyanoacetylhydrazone (compound I)	mg	5000	
Vitamin A	I.U.	1000000	
Vitamin D ₃	I.U.	100000	35
Vitamin B ₂	mg	313	
Vitamin B ₁₂	mg	2	
Vitamin K ₃	mg	200	
Niacin	mg	120	
Methionine	mg	100000	40
Amprol+ethopabate 25% premix	mg	50000	
Ethoxyquine (antioxidant)	mg	12500	

Amprol is a Registered Trade Mark

Upon feeding the supplemented mixture to Tetra SL chickens in the standard feeding test, an increase of the weight gain by 12.6% in comparison to the control group was obtained, and the feed mixture utilization was enhanced by 7.0% over the controls.

In a feed experiment performed on Ross-1 broiler chickens, the above compound I was admixed to the same standard complete feed mixture (BR-1) in doses of 10, 20, 50 and 100 mg/kg of the mixture. (Ross is a Registered Trade Mark). In the course of first 4 weeks of the feeding experiment, the weight gains improved, respectively, by 5.6%, 8.5% (statistically significant), 8.6% (statistically significant), and 5.6% in comparison with the control group. Simultaneously, the feed conversion improved by a margin of from 0.9 to 5.3%.

Example 4

A commercial standard complete feed mixture (COS-2) for feeding early-

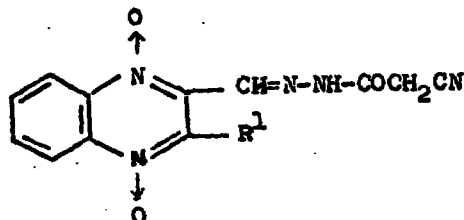
weaned piglets was enriched by admixing to it, in the quantity of 1% by wt., a feed supplement containing specifically active ingredients in the following ratio:

Compound I	mg	5000
Vitamin A	I.U.	800000
Vitamin D ₃	I.U.	200000
Vitamin B ₂	mg	300
Vitamin B ₆	mg	100
Vitamin K ₃	mg	150
Vitamin E	mg	3000
Vitamin C	mg	10000
Niacin	mg	1000
Calcium pantothenate	mg	500
Lysine	mg	50000
Ethoxyquine	mg	12500

Upon feeding the supplemented mixture to early-weaned piglets over 28 days, statistically significant improvement of daily weight gains by 44.1% in comparison with the control group was observed and the feed conversion improved by 20.9% in the first experiment. Similarly, in other analogous parallel trials, the corresponding weight increase and feed conversion improvement values over the controls were 39.9 and 33.7% respectively. Also, in the group fed the supplemented mixture containing the above compound of the invention, not a single case of diarrhoea in piglets following their early weaning occurred and, in both series of the experiments reported, high utility performance of the treated animals was achieved.

WHAT WE CLAIM IS:—

1. 2 - Formylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone of the general formula I

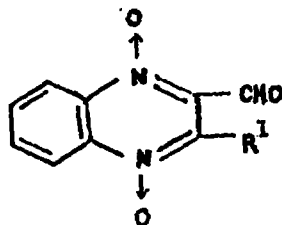


in which R¹ is a hydrogen atom or an alkyl group having 1 to 4 carbon atoms.

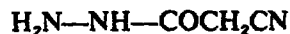
2. 2 - Formylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone.

3. 2 - Formyl - 3 - methylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone.

4. A process for the production of the 2 - formylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone claimed in claim 1, comprising reacting a 2 - formylquinoxaline - 1,4 - dioxide of the general formula II



in which R¹ is as defined in claim 1, or an acetal thereof, with cyanoacetylhydrazine of the formula



in an inert organic or inorganic solvent or diluent or in a mixture thereof, at a temperature of from 0°C to the boiling temperature of the reaction mixture.

5. A process for the production of the compound claimed in claim 1,



1,580,998

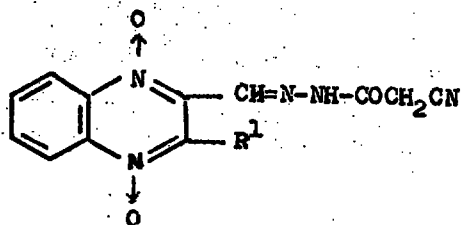
weaned piglets was enriched by admixing to it, in the quantity of 1% by wt., a feed supplement containing specifically active ingredients in the following ratio:

Compound I	mg	5000
Vitamin A	I.U.	800000
Vitamin D ₃	I.U.	200000
Vitamin B ₂	mg	300
Vitamin B ₆	mg	100
Vitamin K ₃	mg	150
Vitamin E	mg	3000
Vitamin C	mg	10000
Niacin	mg	1000
Calcium pantothenate	mg	500
Lysine	mg	50000
Ethoxyquine	mg	12500

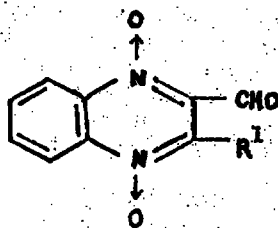
Upon feeding the supplemented mixture to early-weaned piglets over 28 days, statistically significant improvement of daily weight gains by 44.1% in comparison with the control group was observed and the feed conversion improved by 20.9% in the first experiment. Similarly, in other analogous parallel trials, the corresponding weight increase and feed conversion improvement values over the controls were 39.9 and 33.7% respectively. Also, in the group fed the supplemented mixture containing the above compound of the invention, not a single case of diarrhoea in piglets following their early weaning occurred and, in both series of the experiments reported, high utility performance of the treated animals was achieved.

WHAT WE CLAIM IS:—

1. 2 - Formylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone of the general formula I



in which R¹ is a hydrogen atom or an alkyl group having 1 to 4 carbon atoms.
2. 2 - Formylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone.
3. 2 - Formyl - 3 - methylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone.
4. A process for the production of the 2 - formylquinoxaline - 1,4 - dioxide cyanoacetylhydrazone claimed in claim 1, comprising reacting a 2 - formylquinoxaline - 1,4 - dioxide of the general formula II



in which R¹ is as defined in claim 1, or an acetal thereof, with cyanoacetylhydrazine of the formula



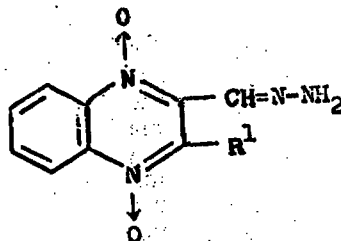
in an inert organic or inorganic solvent or diluent or in a mixture thereof, at a temperature of from 0°C to the boiling temperature of the reaction mixture.
5. A process for the production of the compound claimed in claim 1,



1,580,998

6

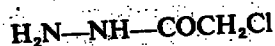
comprising reacting a 2 - formylquinoxaline - 1,4 - dioxide hydrazone of the general formula III



in which R¹ is as defined in claim 1, with a reactive derivative of cyanoacetic acid in an inert organic or inorganic solvent or diluent or mixture thereof, at a temperature from -20 to +100°C.

6. A process as claimed in claim 5, in which the said reactive derivative of cyanoacetic acid is cyanoacetyl chloride or a cyanoacetic acid ester.

7. A process for the production of the compound claimed in claim 1, comprising reacting a 2 - formylquinoxaline - 1,4 - dioxide of the general formula II defined in claim 4 with chloroacetylhydrazine of the formula



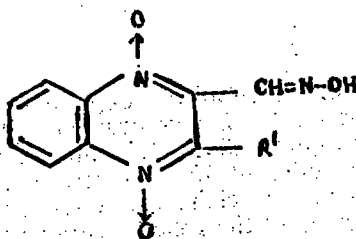
in an inert organic or inorganic solvent or diluent or in a mixture thereof, and thereafter with an alkali metal cyanide.

8. A process for the production of the compound claimed in claim 1, comprising reacting a 2 - formylquinoxaline - 1,4 - dioxide hydrazone of the general formula III defined in claim 5 with chloroacetylchloride in the presence of an organic or inorganic base in an inert organic or inorganic solvent or diluent or in a mixture thereof, and thereafter with an alkali metal cyanide.

9. A process as claimed in claim 8, in which the said base is an alkali metal carbonate or hydrogen carbonate or a tertiary amine.

10. A process as claimed in claim 9, in which the tertiary amine is pyridine or triethylamine.

11. A process for the production of the compound claimed in claim 1, comprising reacting a 2 - formylquinoxaline - 1,4 - dioxide oxime of the general formula IV



in which R¹ is as defined in claim 1, with cyanoacetylhydrazine in an aqueous inorganic acid solution, at a temperature of from 20° to 100°C.

12. A process as claimed in claim 11, in which the aqueous inorganic acid solution is 30 to 98 weight percent sulphuric acid.

13. A composition for use in veterinary medicine or animal nutrition, containing at least one compound of claim 1 as the active principle together with a carrier and/or excipient.

FITZPATRICKS,
Chartered Patent Agents,
14-18 Cadogan Street,
Glasgow, G2 6QW.
and
Warwick House,
Warwick Court,
London, WC1R 5DJ.