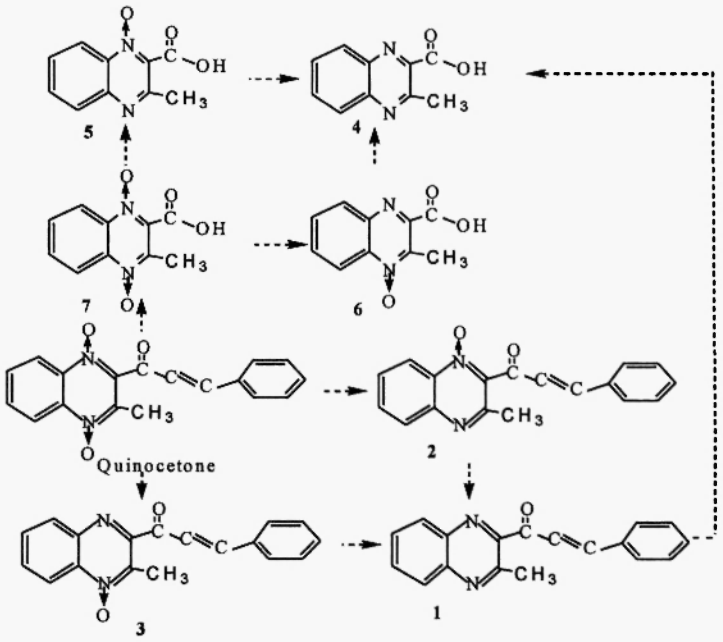
SYNTHESIS OF THE POSSIBLE METABOLITES OF QUINOCETONE IN ANIMALS

Jian-yong Li[1]*, Ji-yu Zhang[1] Xu-zheng Zhou[1], Jin-shan Li[1] and Run-hua Lu[2]
[1]Lanzhou Institute of Animal and Pharmaceutical Veterinary Science,
Chinese Academy of Agricultural Sciences, Lanzhou730050, P.R. China,
[2]Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 460016, P.R. China
email : lijy1971@163.com

Abstract : The possible metabolites of quinocetone in animals had been prepared with different selective reagent by three synthetic routes. It was their principal reaction that $Na_2S_2O_4$ reduced quinoxaline-1,4-dioxide derivatives to quinoxaline derivatives, H_2O_2 $_s$oxidized 2-carboxyl-quinoxaline derivatives to 2-carboxyl-quinoxaline-1-oxide ones and $P(OCH_3)_3$ reduced 2-carboxyl- quinoxaline-1,4-dioxide derivatives to 3-carboxyl-quinoxaline-1-oxide ones. The title compounds ware confirmed with NMR,UV, FAB-MS, et al.

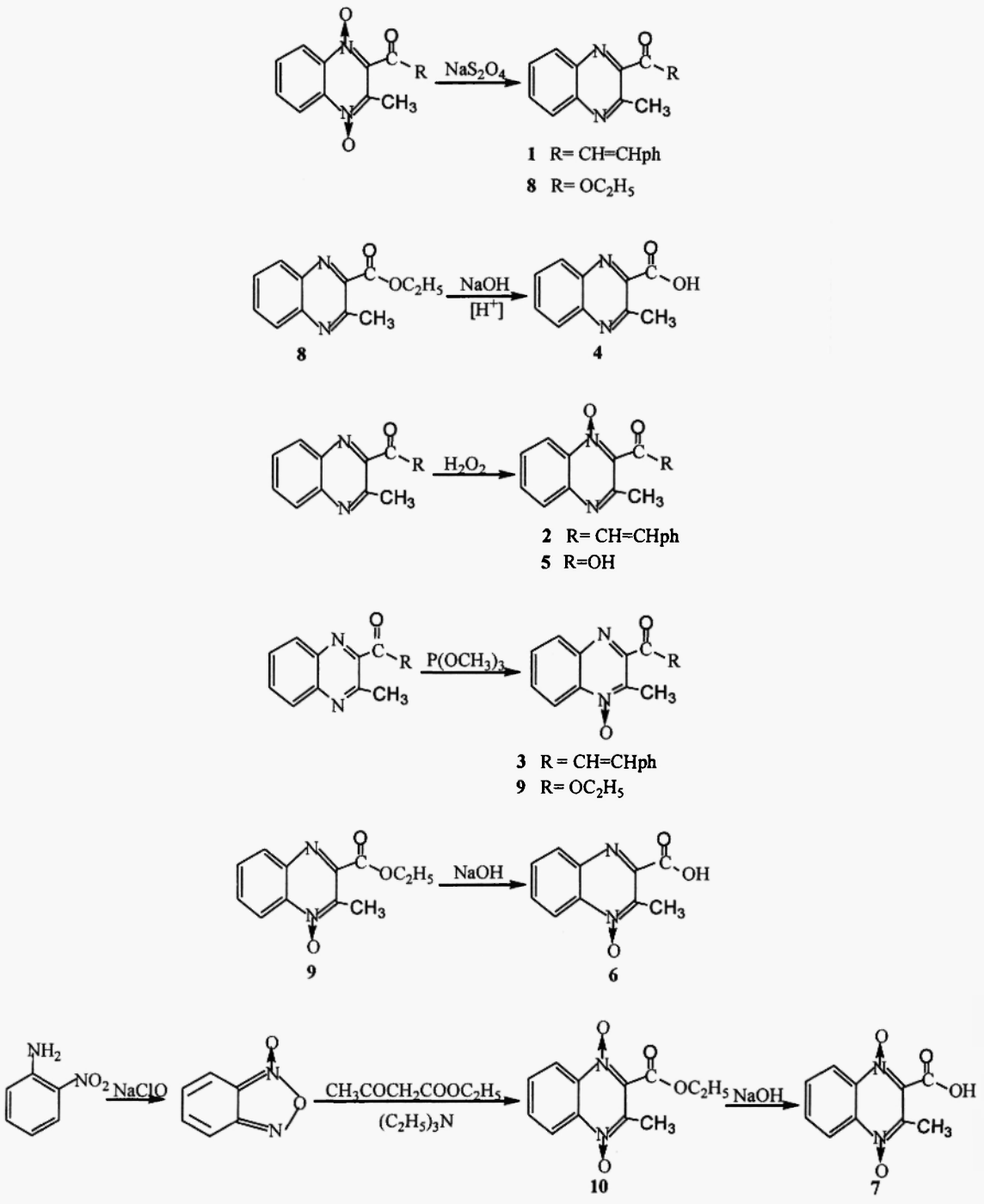
Quinocetone is called 3-methyl-2-cinnamoyl-quinoxaline-1,4-dioxide. It has been used as a novel animal drug and as a feed additive for antibacterial growth promoters. Its pharmacology, toxicology, clinic characters were investigated previously[1-5]. Quinocetone may replace olaquindox and could be applied extensively in poultry and livestock farming. Moreover, Quinocetone (including its parent structure) meets the requirements of modern growth promoters. For low bioavailablity of quinocetone in animals, it is difficult to separate and identify the metabolite of quinocetone from excreta. Compared with the metabolite and structure of olaquindox, the possible metabolites of quinocetone were deduced in chemistry as Scheme-1.

Herein we report on synthesis of these possible metabolites. Two synthetic methods on quinoxalines were frequently reported[6-8]. O-nitroaniline, as starting material, was oxidized to benzofurazan oxide, which reacted with β -diketone or β -keto ester to quinoxaline-1,4-dioxide derivatives. The quinoxaline-1,4- dioxide derivatives was reduced to quinoxaline derivatives. On the other hand, o-phenylenediamine, as starting material, was condensed with α-Br or hydroxyimino substituted-β-diketone or β-keto ester to quinoxaline derivatives, which was oxidized to quinoxaline-1,4-dioxide derivatives. For materials supplied easily from market, the title compounds were prepared by the first synthetic methods.



Scheme-1 : The possible metabolism of quinocetone in animals

Based on their different structures, the synthetic routes of these possible metabolites were divided into three kinds in the following way as Scheme 2. Intermediate compound **10**, synthesized by Beirut reaction, was hydrolyzed to 3-methyl-2-carboxyl-quinoxaline-1,4-dioxide (**7**). $Na_2S_2O_4$ reduced quinoxaline-1,4- dioxide derivatives to quinoxaline derivatives such as synthesis of 3-methyl-2-cinnamoyl-quinoxaline(**1**) and intermediate compound **8**[9]. H_2O_2 and $P(OCH_3)_3$ were selective oxidative and reductive reagents, respectively. H_2O_2 oxidized 2-carboxyl-quinoxaline derivatives to 2-carboxyl-quinoxaline-1-oxide ones such as synthesis of 3-methyl-2-cinnamoyl- quinoxaline-1-oxide (**2**) and 3-methyl-2-carboxyl-quinoxaline-1-oxide (**5**) and $P(OCH_3)_3$ reduced 2-carboxyl- quinoxaline-1,4-dioxide derivatives to 3-carboxyl-quinoxaline-1-oxide ones such as synthesis of 2-methyl-3-cinnamoyl-quinoxaline-1-oxide (**3**) and intermediate compound **9**[10~11]. Intermediate compound **8** and **9** were hydrolyzed to their carboxyl derivatives. The title compounds are confirmed with NMR, UV, FAB-MS, et al. However, all the title compounds were unstable in chemistry, so they should be kept in low temperature, no light and airtight bottle.



Scheme-2 : Synthesis of the possible metabolites of quinocetone

Experimental

Melting points were determined on XT-4 binocular microscope melting point apparatus (Beijing Tech Instrument Co. Ltd.) and are uncorrected. Proton magnetic resonance

(NMR) is determined at 400MHz with a Varian INOVA 400 spectrometer. The chemical shift values are expressed inδvalues relative to the internal standard tetramethylsilane. Mass spectra ware recorded on a ZAB-HS spectrometer. The UV spectra were determined on UV-240 Spectrophotometer (shimadzu).

Preparation of 3-methyl-2-cinnamoyl-quinoxaline (1)

90% $Na_2S_2O_4$ 8.7g (45.0mmol) was added slowly to 11.0g (50mmol) 3-methyl-2-acetyl-quinoxaline-1,4-dioxide in 250ml 95% ethanol with stirring. After refluxed for 3 h, the solution was filtered in heat condition in order to clean the impurity. The solution was cooled, and water was added. The solution was extracted with $CHCl_3$ for 3 times. After the combined $CHCl_3$ was desiccated with $MgSO_4$ and $CHCl_3$ was recycled, recrystallization of the residue with ethanol gave 8.6g(46.1mmol) 3-methyl-2-acetyl-quinoxaline as light yellow solid: m.p.72-74℃.

8.3g (44.6mmol) 3-methyl-2- acetyl-quinoxaline and 6.5g (60.0mmol , 6.5ml) benzaldehyde in 10ml ethanol was churned up in heat condition for 15min. After 3.7g (50.0mmol) diethylamine was poured, the solution was churned up in 35℃ for 3 h. 18 h later, filtration of the solution with 95% ethanol cleanout and dryness gave 4.5g (16.5mmol) 3-methyl-2-cinnamoyl-quinoxaline(1) as yellow solid: m.p. 144-145℃; ^1H NMR (DMSO-d_6) δ 3.00(CH_3),7.41-7.86(Ar-H and CH=CH, 9H), 8.07-8.19 (C_8-H and C_5-H,2H); ^{13}C NMR (DMSO-d_6) δ 23.9 (CH_3),119.9 (C_8), 123.3(C_5), 128.3-153.5(Ar-C and CH=CH,14C), 191.0(C=O); MS (FAB, M+H) 275.2; UV (λ_{max}, nm) 235, 320.

Preparation of 3-methyl-2-cinnamoyl-quinoxaline-1-oxide (2)

A combined mixture of 0.685g (2.5mmol) 3-methyl-2-cinnamoyl-quinoxaline, 2ml trifluoroacetic acid and 0.085 ml (1.26mmol , 50% V/V) hydrogen peroxide were stirred for 1.5 h. After 5ml cold water was added, the solution was neutralized with 40%NaOH. The precipitate was filtered and washed with acetone to 0.3g (1mmol)

3-methyl-2-cinnamoyl-quinoxaline-1-oxide (2) as a yellow solid: m.p.222-224℃; ^{1}H NMR (DMSO-d_6) δ 2.90(CH_3), 6.56-7.71(Ar-H and CH=CH,11H); ^{13}C NMR (DMSO-d_6) δ 17.6 (CH_3), 107.4 -152.3(Ar-C and CH=CH,14C), 154.9(C=O); MS (FAB, M+H) 291.3; UV (λ_{max}, nm) 235, 287, 350, 450.

Preparation of 2-methyl-3-cinnamoyl-quinoxaline-1-oxide (3)

A combined mixture of 6.06g (20.0mmol) 3-methyl-2-cinnamoyl-quinoxaline-1,4- dioxide, 2.87g (22.0mmol) trimethylphosphite and 40ml 1-propanol was heated at reflux for 1.5 h. The mixture was cooled in an ice bath and the precipitate was collected by filtration. recrystallization from ethanol gave 0.8g (2.8mmol) 2-methyl-3-cinnamoyl-quinoxaline-1-oxide (3) as yellow solid: m.p.155-156℃;^{1}H NMR (DMSO-d_6) δ 2.83(CH_3,3H), 7.41-7.81(Ar-H and CH=CH,9H), 8.61(C_8-H, 1H), 8.17(C_5-H,1H); ^{13}C NMR (DMSO-d_6) δ 14.0 (CH_3), 119.0 (C_8), 123.3(C_5), 128.9-151.7(Ar-C and CH=CH,14C), 190.0(C=O); MS (FAB, M+H) 291.3; UV (λ_{max}, nm) 235, 320.

Preparation of 3-methyl-2-carboxyl-quinoxaline (4)

Sodium hypochlorite solution A solution of Sodium hypochlorite was prepared immediately before it was to be used. A mixture of 50g(1.25 moles) of Sodium hydroxide and 200ml of water was stirred until the solid dissolved. The solution was cooled to 0℃, and 100g of crushed ice was added. The flask is then placed in an ice bath, and Chlorine gas from a tank was bubbled through the solution until 41g (0.58mol) was absorbed. An excess of Chlorine should be avoided. The solution of Sodium hypochlorite was kept in the dark at 0℃ until needed.

Benzofurazan oxide (BFO) A mixture of 21g (0.32mole) of potassium hydroxide and 250 ml of 95% ethanol was heated on a steam bath until the solid dissolves. O-nitroaniline

(40g, 0.29mole) was dissolved in the warm alkali solution. The resulting deep red solution was then cooled to 0℃, and the sodium hypochlorite solution was added slowly with good stirring over the course of 10 minutes. The yellow precipitate was collected by filtration, washed with 200ml water, and air-dried. Rectystallization from 95% ethanol gave 32g benzofurazan oxide as yellow solid: m.p.72-73℃.

3-Methyl-2-ethoxylcarbonylquinoxaline-1,4-dioxide A mixture of 21g(0.15mol) BFO and 30g (0.231mol) ethyl acetoacetate in 50ml ethanol was stirred on warm water bath. After 10ml (0.09mol) triethylamine was added, the solution continued to be stirred at 40-45℃ for 2h. When the solution was cooled, the yellow precipitate was collected by filtration, washed with 20ml 95% ethanol and air-dried. Recrystallization from 95% ethanol gave 24.2g(0.098mol) 3-methyl-2-ethoxylcarbonyl-quinoxaline- 1,4-dioxide as yellow solid: m.p. 136-137℃.

3-Methyl-2-ethoxylcarbonylquinoxaline Its preparation method was the same as 1. 3-methyl- 2-ethoxylcarbonylquinoxaline-1,4-dioxide was acted as material. It was white solid and m.p.72-74℃.

3-Methyl-2-carboxyl-quinoxaline (4) A mixture of 6.48g (30.0mmol) 3-methyl-2-ethoxylcarbonyl- quinoxaline-1,4-dioxide, 10ml 40% NaOH and 91ml water was refluxed with stirring for 3h and then bleached with active carbon for 30 minutes. After the active carbon was removed, pH of the solution was adjusted with 1.0M H_2SO_4 to 6 and the precipitate appeared. The precipitate was collected and recrystallized to give 4 as white solid: m.p.158-161 ℃ ; ^{1}H NMR (DMSO-d_6) δ 3.15(CH_3,3H),7.81 (C_6-H,1H),7.90(C_7-H,1H),8.09-8.13(C_8-H and C_5-H,2H); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH_3), 128.7(C_8),129.0(C_5),130.6(C_7),133.0 (C_6), 138.5 (C_9), 139.1(C_{10}),144.0(C_2), 154.9(C_3), 163.2(C=O); MS (FAB, M+H) 189.1; UV (λ_{max}, nm) 235,320.

Preparation of 3-methyl-2-carboxyl-quinoxaline-1-oxide (5)

Its preparation method was the same as 2. 4 was acted as material. It was white solid: m.p.152-153℃; ^{1}H NMR (DMSO-d_6) δ 3.15(CH_3, 3H), 7.80-7.92(C_6-H and C_7-H, 2H), 8.09-8.13(C_8-H and C_5-H,2H); ^{13}C NMR (DMSO-d_6) δ 24.4 (CH_3), 128.6(C_8), 129.0(C_5), 130.6(C_7), 133.0(C_6), 138.5(C_9), 139.1(C_{10}), 144.0(C_2), 154.9(C_3), 163.2(C=O); MS (FAB, M+H) 205; UV (λ_{max}, nm) 235,320.

Preparation of 2-methyl-3-carboxyl-quinoxaline-1-oxide (6)

2-Methyl-3-ethoxyicarbonylquinoxaline-1-oxide Its preparation method was the same 3-methyl-2-ethoxylcarbonylquinoxaline-1,4-dioxide was acted as material. It was white solid: m.p.91-92℃.

2-Methyl-3-carboxyl-quinoxaline-1-oxide (6) Its preparation method was the same as 4. 3-methyl-2-ethoxylcarbonylquinoxaline was acted as material. It was white solid: m.p.142-143 ℃ ; ^{1}H NMR (DMSO-d_6) δ 2.47(CH_3,3H),7.83-7.90(C_6-H and C_7-H,2H),8.11(C_8-H,1H),8.40(C_5-H,1H); ^{13}C NMR (DMSO-d_6) δ 14.0 (CH_3), 118.3(C_8),130.1(C_5),131.5(C_7),131.6(C_6), 136.3(C_9), 138.9(C_{10}),141.9(C_2), 148.5(C_3), 166.1(C=O); MS (FAB, M+H) 205.2; UV (λ_{max}, nm) 235,320.

Preparation of 3-methyl-2-carboxyl-quinoxaline-1,4-dioxide (7)

A mixture of 4.96 g (20.0mmol) 3-methyl-2-ethoxylcarbonylquinoxaline-1,4-dioxide, 100ml 40% NaOH and water was refluxed with stirring for 2.5h and then bleached with active carbon for 30 minutes. After the active carbon was removed, pH of the solution was adjusted with 1.0M H_2SO_4 to 6. The solution was extracted with 500ml $CHCl_3$ for 4 times. After the $CHCl_3$ solution was desiccated with $MgSO_4$ and $CHCl_3$ was recycled, recrystallization of the residue with ethanol gave 7 as white solid: m.p. 221-223℃; ^{1}H NMR (DMSO-d_6) δ 2.41(CH_3,3H),7.40-8.24(C_6-H、C_7-H、C_8-H and C_5-H,4H); ^{13}C NMR (DMSO-d_6) δ 12.3 (CH_3),113.1(C_8 and C_5 ,2C),119.4(C_7 and C_6 ,2C), 123.7(C_9 and C_{10} ,

2C),132.0(C_2 and C_3 , 2C), 152.9(C=O); MS (FAB, M+H) 220.2; UV (λ_{max}, nm) 235,300,359.

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