Synthesis, Structure Elucidation and Antibacterial Activity of 6-Ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylic Acid

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The preparation, structure elucidation and antibacterial activity of 6-ethyl-6,9-dihydro-9-oxopyrazolo-[3,4-*f*]quinoline-8-carboxylic acid are reported.

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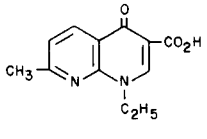
The well-documented antibacterial activity of nalidixic acid (**1**) and oxolinic acid (**2**) has led to the preparation of similar compounds containing the quinoline moiety **3** fused to other heterocyclic groups. Such a compound is 6-ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylic acid (**4**).

Kametani *et al.*, reported the synthesis and antimicrobial evaluation of **4** as well as several related compounds and provided structural proof for these products [1-3]. The present publication reports a synthesis of **4** which was accomplished in these laboratories prior to the Kametani disclosure [4]. Although the two methods of synthesis of **4** are virtually the same, the means of structure elucidation employed in these laboratories differs considerably from those reported by Kametani *et al.*, [1]. The results of this investigation are the subject of this publication.

Reaction of 6-aminoindazole (**5**) with diethyl ethoxymethylenemalonate (**6**) in Dowtherm A gave rise to the ester **7**, the angular structure of which was indicated by the appearance of two doublets at δ 7.77 and 8.33 in the nuclear magnetic resonance spectrum. Conversion to the



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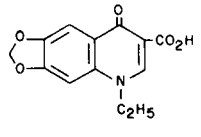

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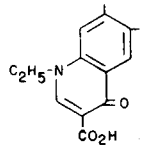

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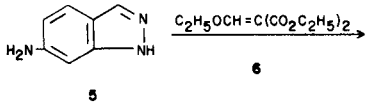
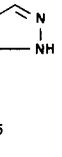
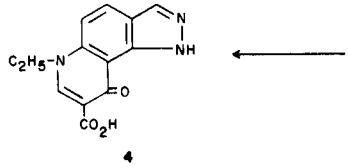
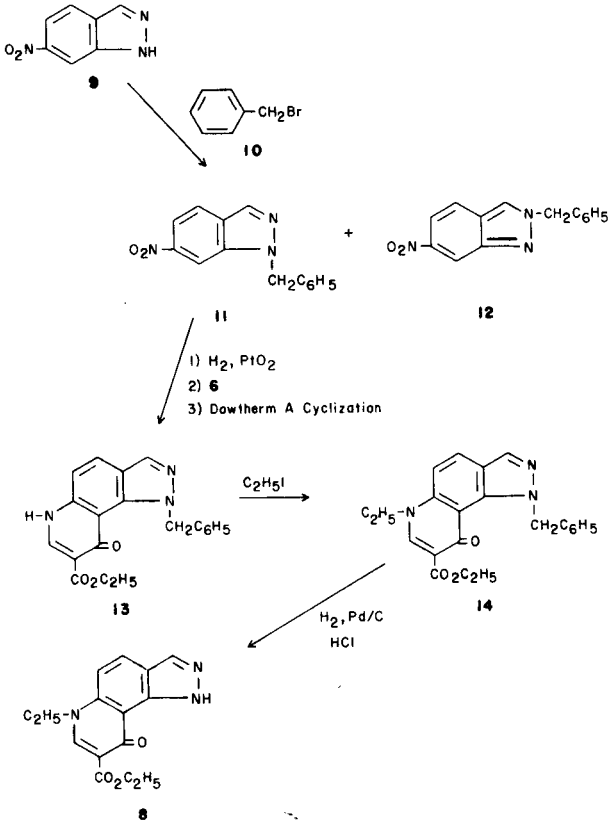


5 **6** **7**



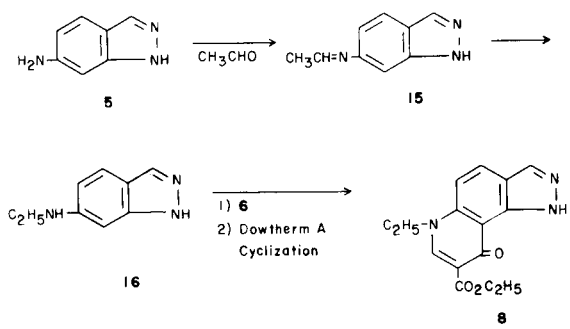


4 **8**



N-ethyl compound **8** was effected with iodoethane in dimethylformamide. Hydrolysis of **8** gave the target carboxylic acid **4** in near-quantitative yield.

To prove that the indazole N-H was not involved in either the ethoxymethylenemalonate step or the ethylation procedure, this function was blocked and the foregoing sequence was carried out. Specifically, treatment of 6-nitroindazole (**9**) with benzyl bromide (**10**) gave a mixture of 1-benzyl-6-nitroindazole (**11**) and 2-benzyl-6-nitroindazole (**12**), which were separated by fractional crystallization. The structures of these products were proven by the superimposibility of their respective ultraviolet spectra with those of the previously reported 1-methyl-6-nitroindazole and 2-methyl-6-nitroindazole [5]. Catalytic hydrogenation of the 1-benzyl compound **11** followed by reaction with **6** and subsequent cyclization in Dowtherm A resulted in ester **13** which upon treatment with iodoethane gave rise to



14. Palladium-on-carbon catalyzed hydrogenolysis of **14** in the presence of hydrogen chloride gave ester **8**, thereby indicating the assigned structure of the latter was correct and the indazole N-H was not involved in the above transformations. The cyclization sequence from **11** to **13** confirms the observations of Kametani *et al.*, [3] who showed that 1-benzyl derivatives of **5** ring closed to the angular pyrazolo[3,4-*f*]quinolines.

Then, to demonstrate that alkylation of **7** gave the *N*-ethyl compound **8** instead of the isomeric *O*-ethyl derivative, the former product was prepared unequivocally as follows: condensation of **5** with acetaldehyde afforded the Schiff base **15** which was converted to the *N*-ethylaminoindazole **16**. Reaction of **16** with **6** and cyclization in Dowtherm afforded the same *N*-ethyl product **8** that was obtained from **7**.

These series of experiments thus verify the structural assignment of **4** as 6-ethyl-6,9-dihydro-9-oxopyrazolo-[3,4-*f*]quinoline-8-carboxylic acid.

Compound **4** was evaluated for *in vitro* activity against a variety of bacterial species according to a serial dilution procedure previously described [6]. The results show that with the exception of *Pseudomonas aeruginosa*, **4** inhibited all the Gram negative species tested at 12.5-25 mcg/ml. This compound was inactive at 25 mcg/ml against *P. aeruginosa* as well as the Gram positive organisms *Staphylococcus aureus* and *Streptococcus agalactiae*.

Compound **4** was administered to rats at 100 mg/kg (50 mg/kg oral and 50 mg/kg ip). The urine collected for 4 hours after dosing inhibited the growth of *Escherichia coli* and *Proteus mirabilis* when diluted four-fold in a glucose-mineral salts medium containing the bacteria.

EXPERIMENTAL

Melting points were taken in a Mel-Temp apparatus in open capillary tubes and are uncorrected. The nuclear magnetic resonance spectra were taken on a Varian-A60A instrument and were compared with TMS as an internal standard. Infrared spectra were determined as Nujol mulls on a Perkin-Elmer 137B spectrophotometer.

Ethyl 6,9-Dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylate (**7**).

A mixture of 108 g (0.50 mole) of diethyl ethoxymethylenemalonate (**6**), 65.5 g (0.50 mole) of 6-aminoindazole (**5**) and 500 ml Dowtherm A was stirred and heated at 235-242° for 6.5 hours and the ethanol evolved was collected in a Dean-Stark apparatus (volume collected: 55.0 ml, theoretical amount: 58.5 ml). The mixture was allowed to cool to 80°, diluted with 1000 ml heptane, and stirred for 30 minutes. Filtration gave 99 g of the crude product. Recrystallization from 2400 ml dimethylformamide gave, in two crops, 48.6 g (38%) of the product, mp 309-311°. Further recrystallization from dimethylformamide gave an analytical sample, mp 318-320°, mp lit [1], 299-302° dec; nmr (trifluoroacetic acid): δ 1.55 (t, 3, CH_3CH_2O), 4.56 (q, 2, CH_3CH_2O), 7.77, 8.33 (2d, 2, J = 9 Hz, C_4-H, C_5-H) and 8.61, 9.08 (2s, 2, C_3-H and C_7-H); ir: μ 2.97 (N-H), 5.83 (C=O, ester), 6.08-6.13 (C=O, lactam), 6.23 (C=C), 8.28-8.36 (C-O-C, ester).

Anal. Calcd. for $C_{13}H_{11}N_3O_3$: C, 60.69; H, 4.31; N, 16.74. Found: C, 60.45; H, 4.47; N, 16.39.

Ethyl 6-Ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylate (**8**).

A mixture of 33.4 g (0.13 mole) of **7**, 17.94 g (0.13 mole) of potassium carbonate and 20.28 g (0.13 mole) of iodoethane in 500 ml DMF was stirred at 57-63° for 60 hours, cooled to 25°, and poured into 2000 ml cold stirred tap water. The solution was stirred for 20 minutes and extracted with 2 × 400 ml of chloroform. The combined extracts were dried (magnesium sulfate) and concentrated to dryness *in vacuo* to give 28.8 g of the crude product. The solid was boiled with 600 ml ethanol and the suspension was refrigerated for 18 hours. Filtration, washing with 100 ml ethanol, air drying, and drying at 60° gave 21.70 g (59%) of the product, mp 230-234°. An analytical sample, mp 231-235°, was obtained by recrystallization from ethanol, mp lit [1], 238-239°; nmr (trifluoroacetic acid): δ 1.53, 1.78 (2t, 6, CH_3CH_2O and CH_3CH_2N), 4.60, 4.90 (2q, 4, CH_3CH_2O and CH_3CH_2N), 7.90, 8.52 (2d, 2, J = 9 Hz, C_4-H, C_5-H) and 8.68 and 9.19 (2s, 2, C_3-H and C_7-H); ir: μ 2.90 (N-H), 5.90 (C=O, ester), 6.09 (C=O, lactam), 6.20 (C=C), and 8.27-8.32 (C-O-C, ester).

Anal. Calcd. for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.03; H, 5.31; N, 14.63.

6-Ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylic Acid (**4**).

A mixture of 11.40 g (0.04 mole) of **8** and 400 ml 10% aqueous potassium hydroxide was stirred and refluxed for 4.0 hours, filtered while hot through glass wool, and acidified to *p*H 5 with 6*N* aqueous hydrochloric acid at 60°. The mixture was stirred at ambient temperature for 30 minutes and the solid was filtered, washed with 2 × 50 ml water, air dried, and dried at 60° for 18 hours to give 10.0 g (97%) of the product, mp 315-318° dec. An analytical sample, mp 312-315°, was obtained by recrystallization from dimethylformamide, mp lit [1], >300°; nmr (trifluoroacetic acid): δ 1.80 (t, 3, CH_3CH_2N), 4.88 (q, 2, CH_3CH_2N), 7.60, 8.53 (2d, 2, J = 9 Hz, C_4-H and C_5-H), 7.70, 9.23 (2s, 2, C_3-H and C_7-H); ir: μ 3.00 (N-H), 5.76-5.81 (C=O, acid), 6.10 (C=O, lactam) and 6.19 (C=C).

Anal. Calcd. for $C_{13}H_{11}N_3O_3$: C, 60.69; H, 4.31; N, 16.34. Found: C, 60.31; H, 4.24; N, 16.41.

1-Benzyl-6-nitroindazole (**11**) and 2-Benzyl-6-nitroindazole (**12**).

To a solution of 16.3 g (0.100 mole) of 6-nitroindazole (**9**) in 100 ml dimethylformamide was added quickly 5.28 g of sodium hydride – 60% in mineral oil (3.17 g, 0.13 mole), the reaction temperature being maintained below 45° with a water bath. Benzyl bromide (**10**) (17.1 g, 0.10 mole) was added quickly with a water bath being employed to maintain the temperature between 30-40°. The mixture was stirred at 90-100° for 4 hours, cooled and poured into 600 ml tap water. The solid was filtered and dried to give 25 g of the crude product. The material was dissolved in 300 ml boiling toluene, decolorized, and the solution was concentrated to dryness *in vacuo*. Recrystallization from 100 ml ethanol gave 16.70 g of a mixture of **11** and **12**. The solid was dissolved in 115 ml boiling toluene and after cooling there was deposited 3.18 g (12%) of the 2-benzyl compound **12**. Further recrystallization from toluene gave an analytical sample, mp 121-123°; nmr (DMSO-d_6): δ 5.80 (s, 2, $C_6H_5CH_2$), 7.42 (s, 5, $C_6H_5CH_2$), 7.92 (m, 2, aromatic C-H), 8.66 (m, 1, aromatic C-H), 8.78 (s, 1, indazole 3-CH); ir: μ 6.40 (C=C), 6.59 and 7.50 (NO_2).

Anal. Calcd. for $C_{14}H_{11}N_3O_2$: C, 66.39; H, 4.38; N, 16.59. Found: C, 66.40; H, 4.29; N, 16.62.

The filtrate from the isolation of **12** was concentrated to dryness and the residue was recrystallized from 100 ml ethanol to give 4.47 g (18%) of **11**. Further recrystallization from ethanol gave an analytical sample, mp 106-107°; nmr (DMSO-d_6): δ 5.63 (s, 2, $C_6H_5CH_2$), 7.03 (s, 5, $C_6H_5CH_2$), 7.73, 8.03, 8.43 (3s, 4, aromatic C-H); ir: μ 6.29 (C=C), 6.54 and 7.40 (NO_2).

Anal. Calcd. for $C_{14}H_{11}N_3O_2$: C, 66.39; H, 4.38; N, 16.59. Found: C, 66.44; H, 4.43; N, 16.23.

The ultraviolet spectra of **11** and **12** in 95% ethanol were identical to those of 1-methyl 6-nitroindazole and 2-methyl-6-nitroindazole, respectively, which were synthesized by the literature method [5].

Ethyl 1-Benzyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylate (**13**).

A mixture of 50.6 g (0.200 mole) of **11**, 1.0 g platinum dioxide (Engelhard) and 650 ml methanol was shaken with hydrogen for 24 hours, uptake hydrogen 30.5 lbs. The catalyst was filtered and washed with 2 × 100 ml of methanol. To the filtrate and combined washings was added another 1.0 g platinum dioxide. Upon shaking for 2.5 hours another 2.6 lbs. of hydrogen was consumed, total uptake 33.1 lbs.; theoretical: 40.8 lbs. The catalyst was filtered and washed with 2 × 50 ml methanol. The filtrate and combined washings were concentrated to dryness *in vacuo* and the residue was dissolved in 500 ml Dowtherm A. To the solution was added 43.2 g (0.200 mole) diethyl ethoxymethylenemalonate (**6**). The mixture was stirred and heated to 245° over a two-hour period and then heated at 245° for two hours, the ethanol was collected in a Dean-Stark apparatus, amount collected 17.5 ml, theoretical amount: 23.4 ml. The mixture was cooled to 60° and diluted with 1000 ml of heptane. After stirring for 18 hours the solid was filtered, air dried, and dried at 60° to give 44 g of crude product. Recrystallization from ethanol gave in four crops 34.4 g (50%) of the product, mp 272-277°. An analytical sample, mp 280-282°, mp lit [3], 279-282°, was obtained by recrystallization from dimethylformamide; nmr (trifluoroacetic acid): δ 1.36 (t, 3, CH_3CH_2O), 4.32 (q, 2, CH_3CH_2O), 6.53 (s, 2, $C_6H_5CH_2N$), 7.16 (s, 5, $C_6H_5CH_2$), 7.36, 8.07 (2d, 2, J = 9 Hz, C_4-H and C_5-H), 8.33, 8.56 (2s, 2, C_3-H and C_7-H); ir: μ 5.85-5.91 (C=O, ester), 6.11, 6.20 (C=C), 8.40-8.50 (C-O-C, ester).

Anal. Calcd. for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10. Found: C, 68.79; H, 4.94; N, 11.78.

Ethyl 1-Benzyl-6-ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylate (**14**).

A mixture of 21.70 g (0.0625 mole) of **13**, 9.66 g (0.07 mole) of potassium carbonate, and 10.92 g (0.02 mole) of iodoethane in 220 ml dimethylformamide was stirred at 55-65° for 24 hours and then heated at 90° for 3.5 hours. After cooling, the solution was poured into 1400 ml cold water and the mixture was stirred for two hours. The solid was filtered, washed with 2 × 100 ml water, and air dried to give 26 g of the crude product. Recrystallization from acetonitrile gave, in two crops, 14.3 g (61%) of the product, mp 157-164°. An analytical sample, mp 157-159°, was obtained by recrystallization from acetonitrile; nmr (DMSO-d_6): δ 1.30, 1.32 (2t, 6, CH_3CH_2O and CH_3CH_2N), 4.13 (2q, 4, CH_3CH_2O and CH_3CH_2N), 6.12 (s, 2, $C_6H_5CH_2N$), 6.83 (s, 5, $C_6H_5CH_2$), 7.20, 7.82 (2d, 2, J = 9 Hz, C_4-H and C_5-H), 8.00, 8.32 (2s, 2, C_3-H and C_7-H); ir: μ 5.80 (C=O, ester), 6.10 (C=O, lactam), 6.21 (C=C), 8.40 (C-O-C, ester).

Anal. Calcd. for $C_{22}H_{21}N_3O_3$: C, 70.38; H, 5.64; N, 11.19. Found: C, 70.30; H, 5.69; N, 11.08.

Ethyl 6-Ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylate (**8**) (from **14**).

A mixture containing 0.70 g (0.0019 mole) of **14**, 2.0 g 5% palladium on carbon (50% moisture), and 150 ml of ethanolic hydrogen chloride was shaken with hydrogen on a Parr apparatus at 3-4 atmospheres for 23 hours. The catalyst was filtered and washed with 3 × 15 ml of absolute ethanol followed by 3 × 15 ml dimethylformamide. The ethanolic filtrates were combined, dissolved in the dimethylformamide wash solutions and the resulting solution was poured into 250 ml of a 2% potassium carbonate/water solution. The mixture was stirred for 5 minutes and extracted with 2 × 125 ml chloroform. The combined extracts were dried (magnesium sulfate), concentrated to dryness, and the residue was recrystallized from ethanol to give, in two crops, 0.24 g (44%) of the product which exhibited infrared and nuclear magnetic resonance spectra identical to those of the ester **8** which was derived from **5**.

N-Ethylideneindazol-6-amine (**15**).

To a solution of 20.0 g (0.150 mole) of 6-aminoindazole **6** in 500 ml methanol was added at 25-30° over six minutes 8.80 g (0.200 mole) of acetaldehyde. The mixture was stirred at ambient temperature for 1.5 hours and the solid was filtered, washed with 100 ml methanol, and dried at 60° to give 22 g (92%) of the product **15**, mp 182-184°; ir: μ 3.00, 3.07 (N-H), 6.10 (C=N).

Anal. Calcd. for $C_9H_9N_3$: C, 67.90; H, 5.70; N, 26.40. Found: C, 67.68; H, 5.79; N, 26.07.

6-Ethylaminoindazole (**16**).

A mixture containing 22 g (0.138 mole) of **15**, 2.0 g 5% palladium on carbon, 0.5 ml ethanolic hydrogen chloride, and 1.1 ℓ of methanol was shaken with hydrogen at 3-4 atmospheres for 24 hours. The mixture was heated, decolorized, and filtered. The filtrate was concentrated to dryness *in vacuo* to give 20 g of the crude product, mp 122-138°. Recrystallization from 30 ml of acetonitrile gave 6.0 g (27%) of **16**, mp 113-117°. Further recrystallization from methanol gave an analytical sample, mp 115-120°; nmr (DMSO-d_6): δ 1.18 (t, 3, CH_3CH_2N), 2.82-3.26 (m, 2, CH_3CH_2N), 5.63 (exchangeable broad t, 1, CH_3CH_2NH-), 6.35 (s, 1, indazole 7-CH), 6.52 (d, 1, J = 8 Hz, indazole 5-CH), 7.37 (d, 1, J = 8 Hz, indazole 4-CH), 7.73 (s, 1, indazole 3-CH), 12.3 (exchangeable broad m, 1, indazole 1-NH); ir: μ 3.00, 3.11 (N-H), 6.12 (C=N).

Anal. Calcd. for $C_9H_{11}N_3$: C, 67.06; H, 6.88; N, 26.07. Found: C, 67.04; H, 6.96; N, 25.89.

Ethyl 6-Ethyl-6,9-dihydro-9-oxopyrazolo[3,4-*f*]quinoline-8-carboxylate (**8**) (from **16**).

A mixture of 1.61 g (0.01 mole) of **16**, 2.16 g (0.01 mole) of **6**, and 50 ml Dowtherm A was heated at 255° for 2 hours, cooled and diluted with 200 ml heptane. The insoluble material was dissolved in 200 ml chloroform. The solution was decolorized and concentrated to dryness to give a residue which was extracted with toluene. The toluene extract was concentrated to dryness and the residue was slurried with ethanol to give 0.06 g (2%) of **8**, the infrared spectrum of which was identical to that of the product obtained by alkylation of **7**.

Acknowledgement.

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