SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 3-OXO-3*H*--PYRIDO[3,2,1-*kl*]PHENOXAZINE-2-CARBOXYLIC ACIDS

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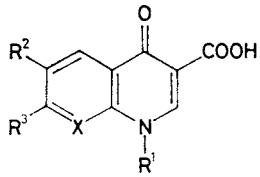
Reaction of ethyl 1,4-dihydro-8-hydroxy-4-oxo-3-quinolinecarboxylates *XIIIa*—*XIIIc* with 2,4--dinitrochlorobenzene in N,N-dimethylformamide in the presence of sodium hydrogen carbonate yields appropriate ethyl 3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylates *XIVa*—*XIVc*. Similar reaction of *XIIIa* and *XIIIb* in aqueous solution yields mixtures of the respective ester (*XIVa* and *XIVb*, respectively) and acid (*XIVd* and *XIVe*, respectively). Compounds *XIVg*—*XIVj* were prepared by usual methods. The compounds prepared were tested for their antimicrobial activity in vitro.

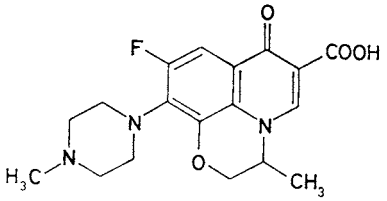
Many compounds with a 4-pyridone-3-carboxylic acid fragment have been studied and evaluated as potential antibacterial agents during the last 25 years[1-3]. First generation drugs, e.g. nalidixic acid (*Ia*), and oxolinic acid (*Ib*), exhibit activity against most gram-negative bacteria but inactivity against *Pseudomonas aeruginosa* and gram-positive bacteria. Second generation drugs are active also against *P. aeruginosa*. Third generation quinolones have greater activity against gram-negative bacteria including *P. aeruginosa*, and, in addition, are highly active against most gram--positive organisms[2,3]. The third generation agents, like norfloxacin (*Ic*), pefloxacin (*Id*), ciprofloxacin (*Ie*), and ofloxacin (*II*), are fluorocompounds with a piperazinyl



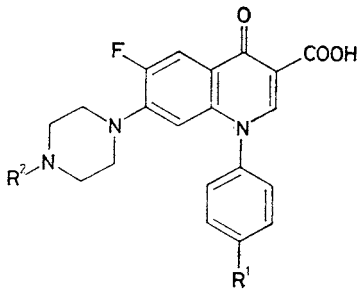
Ia, X = N; R^1 = C_2H_5; R^2 = H; R^3 = CH_3
Ib, X = CH; R^1 = C_2H_5; R^2,R^3 = —CH_2—O—CH_2—
Ic, X = CH; R^1 = C_2H_5; R^2 = F; R^3 = 1-piperazinyl
Id, X = CH; R^1 = C_2H_5; R^2 = F; R^3 = 4-methyl-1-piperazinyl
Ie, X = CH; R^1 = cyclopropyl; R^2 = F; R^3 = 1-piperazinyl

substituent. Structure activity studies seemed to indicate that the antibacterial activity is greatly influenced by the steric bulk of the N-1 substituent[1-4]. The most potent

agents are ethyl-substituted compounds, or compounds having a group with a similar steric bulk at this position. Ofloxacin (*II*), one of the most promising drug of this type, has a 1,8-bridge with a similar steric demand in the crucial area. Recently some very potent 1-aryl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids of a geneal formula *III* have been developed[5-7]. The high activity of this class of antibacterial

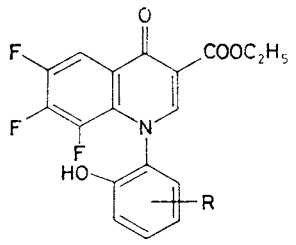


II

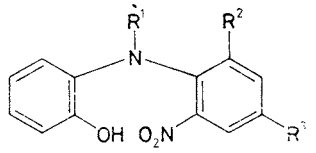


III, R^1 = F, OH ; R^2 = H, CH_3

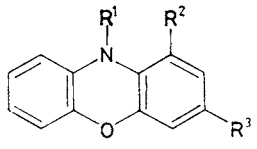
agents incited us to prepare similar rigid structures. In this paper synthesis and antibacterial activity of some 3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylic acids are described. An independent alternate synthesis of this type of compounds has appeared recently[8-10]. Their synthetic pathway to the desired compounds includes an intramolecular nucleophilic displacement cyclization reaction of 6,7,8-trifluoro--1,4-dihydro-1-(2-hydroxyphenyl)-4-oxo-3-carboxylates (*IV*), prepared from 2,3,4,5--tetrafluorobenzoic acid in several steps.



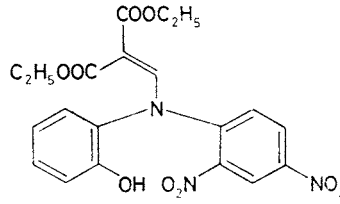
IV



V, R^1 = H, alkyl ; R^2 and/or R^3 = NO_2

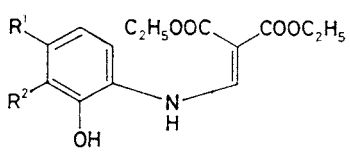


VI, R^1 = H, alkyl ; R^2 and/or R^3 = NO_2

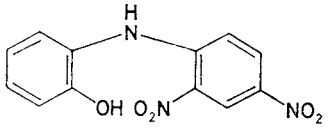


VII

Certain 2-hydroxy-2′-nitrodiphenylamines with another electron-attracting group at the 4′ or 6′ position of a general formula *V*, especially N-substituted ones, undergo an intramolecular denitrocyclization reaction in alkaline conditions. This reaction was first used by Turpin[11] for the preparation of nitrophenoxazines *VI*. The reaction is performed either in an aqueous alkaline solution[11] or the appropriate sodium phenolate is heated in a nonpolar aprotic solvent[12]. For this purpose we tried to prepare diethyl N-(2-hydroxyphenyl)-N-(2,4-dinitrophenyl)aminomethylenemalonate (*VII*) from diethyl N-(2-hydroxyphenyl)aminomethylenemalonate (*VIIIa*) and 2,4-dinitrochlorobenzene. 2-Hydroxy-2′,4′-dinitrodiphenylamine (*IX*) was the only product of this reaction. This compound failed to react with diethyl ethoxymethylenemalonate under usual conditions.

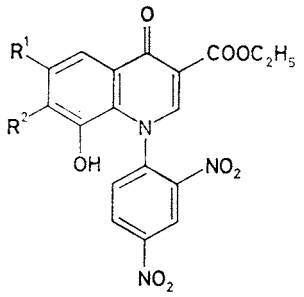


VIIIa, $R^1 = R^2 = H$
VIIIb, $R^1 = H$; $R^2 = Cl$
VIIIc, $R^1 = R^2 = F$

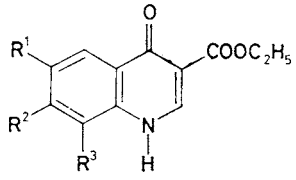


IX

Then we decided to apply the Turpin reaction to compounds of a general formula *X*, in which the diphenylamine structure is masked in the 1-arylquinolone skeleton. N-Alkylation of 1,4-dihydro-4-oxo-3-quinolinecarboxylates is a well established method which has been used for many years[1]. However, no arylation of these compounds has been reported. Earlier we managed to arylate ethyl 1,4-dihydro-6-methoxy-7-(2-methoxyethoxy)-4-oxo-3-quinolinecarboxylate (*XIa*) with 2,4-dinitro-

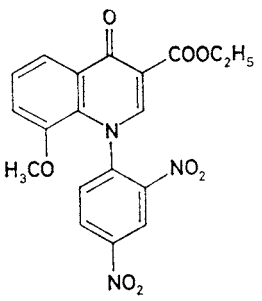


Xa, $R^1 = R^2 = H$
Xb, $R^1 = H$; $R^2 = Cl$
Xc, $R^1 = R^2 = F$

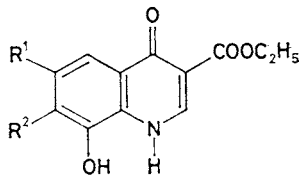


XIa, $R^1 = CH_3O$; $R^2 = CH_3OCH_2CH_2O$; $R^3 = H$
XIb, $R^1 = R^2 = H$; $R^3 = CH_3O$

chlorobenzene in N,N-dimethylformamide after deprotonation with sodium hydride[13]. Similar arylation of ethyl 1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylate (*XIb*, ref.[14]) yielded ethyl 1,4-dihydro-8-methoxy-1-(2,4-dinitrophenyl)-4-oxo-3-quinolinecarboxylate (*XII*) in good yields. Attempts to prepare *Xa* by demethylation of this compound by aluminium chloride, pyridine hydrochloride and/or hydrobromic acid led to a fission of the bond between N-1 and 2,4-dinitrophenyl ring. Therefore we tried to prepare compounds *Xa*–*Xc* by a direct arylation of the respective 8-hydroxyderivatives *XIIIa*–*XIIIc*. Compound *XIIIa* was prepared

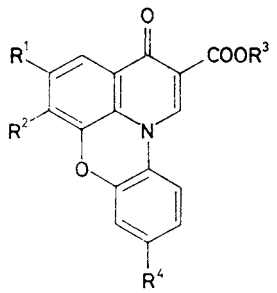


XII



XIII a, $R^1 = R^2 = H$
XIII b, $R^1 = H$; $R^2 = Cl$
XIII c, $R^1 = R^2 = F$

according to the described method[15,16] from 2-aminophenole via diethyl N-(2-hydroxyphenyl)aminomethylenemalonate *VIIIa*. Similarly compounds *XIIIb* and *XIIIc* were prepared. A complex dark mixture was obtained as a result of the reaction of *XIIIa* with 2,4-dinitrochlorobenzene in N,N-dimethylformamide after deprotonation with one molar equivalent of sodium hydride. Reaction of compounds *XIIIa* to *XIIIc* with 2,4-dinitrochlorobenzene in N,N-dimethylformamide in the presence of sodium hydrogen carbonate yielded directly ethyl 3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylates *XIVa*–*XIVc*. The nitro group from the proposed intermediates *Xa*–*Xc* was lost as a nitrite ion by an intramolecular nucleophilic attack. No by-product in significant amount was detected in reaction mixture (TLC, Silufol UV 254, benzene–ethanol 9 : 1). Yields were not improved by performing the reaction under nitrogen atmosphere. Esters *XIVa*–*XIVc* were hydrolyzed to the appropriate acids *XIVd*–*XIVf* by refluxing with a mixture of hydrochloric and acetic acids. Reaction of compound *XIIIa* with an excess of 2,4-dinitrochlorobenzene in aqueous–ethanolic solution in the presence of sodium hydrogen carbonate yielded a mixture of *XIVa* and *XIVd*. After refluxing the reaction mixture for 8 h, only the acid *XIVd* was obtained. Reaction mixture contained also 2,4-dinitrophenole and 2,4-dinitro-1-ethoxybenzene (TLC, Silufol UV 254, benzene–ethanol 9 : 1). Compound *XIVe* was prepared by a similar way, the obtained mixture of *XIVb* and *XIVe* was hydrolyzed under acidic conditions. We failed to prepare compound *XIVf* by this reaction in aqueous-ethanolic solution.



XIV a, $R^1 = R^2 = H$; $R^3 = C_2H_5$; $R^4 = NO_2$
XIV b, $R^1 = H$; $R^2 = Cl$; $R^3 = C_2H_5$; $R^4 = NO_2$
XIV c, $R^1 = R^2 = F$; $R^3 = C_2H_5$; $R^4 = NO_2$
XIV d, $R^1 = R^2 = R^3 = H$; $R^4 = NO_2$
XIV e, $R^1 = H$; $R^2 = Cl$; $R^3 = H$; $R^4 = NO_2$
XIV f, $R^1 = R^2 = F$; $R^3 = H$; $R^4 = NO_2$
XIV g, $R^1 = NO_2$; $R^2 = Cl$; $R^3 = H$; $R^4 = NO_2$
XIV h, $R^1 = F$; $R^2 =$ 4-methyl-1-piperazinyl; $R^3 = H$; $R^4 = NO_2$
XIV i, $R^1 = R^2 = F$; $R^3 = H$; $R^4 = NH_2$
XIV j, $R^1 = F$; $R^2 =$ 4-methyl-1-piperazinyl; $R^3 = H$; $R^4 = NH_2$

Nitration of compound *XIVe* by a mixture of nitric and sulfuric acids yielded *XIVg*. Compound *XIVf* was reduced to *XIVi* by ferrous sulfate under alkaline conditions. Upon a treatment of *XIVf* and *XIVi* with 4-methylpiperazine in pyridine a displacement reaction proceeded at C-6 to give *XIVh* and *XIVj*, respectively. We failed to perform this displacement reaction with 6-chloro derivatives *XIVe* and *XIVg*.

Compounds *XIVc*–*XIVj* were tested for their antimicrobial activity in vitro at the Department of Microbiology of the Institute (Head: Dr V. Hola). The minimum inhibitory concentrations in mg/l are given unless they exceed 128 mg/l: *Streptococcus pyogenes*, *XIVc* 64, *XIVe* 32, *XIVh* 16, *XIVi* 32, *XIVj* $\leqq$ 1; *Streptococcus faecalis*, *XIVd* 64, *XIVf* 64, *XIVh* 8, *XIVi* 32, *XIVj* 8; *Staphylococcus aureus*, *XIVd* 32, *XIVf* 32, *XIVh* 32, *XIVi* 16, *XIVj* 16; *Escherichia coli*, *XIVd* 32, *XIVf* 128, *XIVh* 32, *XIVi* 16, *XIVj* 16; *Pseudomonas aeruginosa*, *XIVd* 32, *XIVf* 128, *XIVg* 128, *XIVi* 64, *XIVj* 16; *Proteus vulgaris*, *XIVd* 64, *XIVh* 64, *XIVi* 16, *XIVj* 16. The low activity or inactivity of the 9-nitro derivatives *XIVd*–*XIVg* is probably due to their very low water-solubility.

EXPERIMENTAL

The melting points were determined on a Mettler FP 5 apparatus, those exceeding 300°C were determined on a Kofler block, and were not corrected. IR spectra were taken on a Unicam SP-2006 spectrometer in KBr pellets; wavenumbers are given in cm^{-1}. UV spectra were taken on a Unicam PU 8 800 spectrophotometer in ethanol, molar absorption coefficients (ε) are given

in m^2 mol^{-1}, wavelengths (λ) in nm. Mass spectra were measured on MCH 1 320 and MAT 44 S spectrometers. ^1H NMR spectra were measured on an apparatus BS-567 A (Tesla Brno) 100 MHz; the standard was pentadeuterated 3-trimethylsilylpropionic acid. The spectral data and elemental analyses were consistent with the assigned structures.

2-Hydroxy-2',4'-dinitrodiphenylamine (*IX*)

A solution of sodium hydrogen carbonate (2·5 g, 300 mmol) in water (25 ml) was added to a solution of diethyl N-(2-hydroxyphenyl)aminomethylenemalonate *VIIIa* (2·79 g, 10 mmol) and 2,4-dinitrochlorobenzene (2·02 g, 10 mmol) in ethanol (25 ml) and the mixture was refluxed for 4 h. Then water was added (25 ml), the mixture was cooled and the separated crystals were filtered off and crystallized from ethanol; yield 2·1 g (76%), m.p. 196·5—201·1°C. Ref.[17] reports m.p. 198—199°C. For $C_{12}H_9N_3O_5$ (275·2) calculated: 52·37% C, 3·30% H, 15·27% N; found: 52·10% C, 3·32% H, 15·09% N. IR spectrum: 3 280 (OH); 3 200 (NH); 1 620, 1 530, 1 490 (arom. system); 1 580 (NO_2). Mass spectrum *m/z*: 275 (M^+).

Ethyl 1,4-Dihydro-1-(2,4-dinitrophenyl)-8-methoxy-4-oxo-3-quinolinecarboxylate (*XII*)

Sodium hydride (80% dispersion in mineral oil, 0·33 g, 11 mmol) was added to a stirred suspension of ethyl 1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylate[14] (*XIb*; 2·47 g, 10 mmol) in N,N-dimethylformamide (25 ml) and the mixture was refluxed for 4 h. Then a solution of 2,4-dinitrochlorobenzene (2·02 g, 10 mmol) in N,N-dimethylformamide (5 ml) was added and the mixture was refluxed for 4 h. The mixture was poured into water (200 ml), the insoluble part was filtered off and crystallized from ethanol; yield 2·2 g (53%), m.p. 185·2—186·0°C. For $C_{19}H_{15}N_3O_8$ (413·3) calculated: 55·21% C, 3·66% H, 10·17% N; found: 54·93% C, 3·49% H, 10·14% N. IR spectrum: 1 705 (CO, COO); 1 610, 1 495, 1 480 (arom. system); 1 535 (NO_2); 1 280 (—O—). UV spectrum: λ_{max} 208 (log ε 3·61), 254 (3·70).

Diethyl N-(3-Chloro-2-hydroxyphenyl)aminomethylenemalonate (*VIIIb*)

A mixture of 2-chloro-6-aminophenole[18] (43 g, 0·3 mol), diethyl ethoxymethylenemalonate (64·8 g, 0·3 mol) and ethanol (75 ml) was refluxed for 1 h, cooled down and left to stand overnight in a refrigerator. The separated crystals were filtered off and crystallized from ethanol; yield 88 g (93%), m.p. 166·4—168·2°C. For $C_{14}H_{16}ClNO_5$ (313·7) calculated: 53·60% C, 5·14% H, 11·30% Cl, 4·46% N; found: 53·26% C, 5·07% H, 11·58% Cl, 5·07% N.

Ethyl 1,4-Dihydro-7-chloro-8-hydroxy-4-oxo-3-quinolinecarboxylate (*XIIIb*)

A mixture of *VIIIb* (31·4 g, 0·1 mol) and diphenyl ether (500 g) was stirred under reflux for 10 min, and, after cooling, hexane (1 l) was added. The separated solid was filtered off, washed with hexane and crystallized from ethanol; yield 18·3 g (68%), m.p. 236·2—237·9°C. For $C_{12}H_{10}ClNO_4$ (267·7) calculated: 53·85% C, 3·77% H, 13·25% Cl, 5·23% N; found 53·71% C, 3·87% H, 13·07% Cl, 4·91% N. IR spectrum: 3 300 (OH); 3 160 (NH); 1 710 (COO); 1 690 (CO), 1 630, 1 550, 1 530 (arom. system).

Diethyl N-(3,4-Difluoro-2-hydroxyphenyl)aminomethylenemalonate (*VIIIc*)

A mixture of 2,3-difluoro-6-nitrophenole[19] (28·1 g, 0·16 mol), ethanol (350 ml) and a spoonful of Raney nickel was reduced with hydrogen (11 l) without pressure, Raney nickel was filtered off and the filtrate was taken to dryness. The dark oil (17·5 g) was dissolved in a mixture of ethanol

(90 ml) and diethyl ethoxymethylenemalonate (26·1 g, 0·12 mol) and the mixture was refluxed for 1 h, cooled and left to stand overnight in a refrigerator. The separated dark crystals were filtered off, washed with benzene and crystallized from 2-propanol; yield 22·6 g (45%), m.p. 177·7—179·2°C. For $C_{14}H_{15}F_2NO_5$ (315·3) calculated: 53·34% C, 4·80% H, 12·05% F, 4·44% N; found: 53·06% C, 4·85% H, 12·44% F, 4·28% N.

Ethyl 1,4-Dihydro-6,7-difluoro-8-hydroxy-4-oxo-3-quinolinecarboxylate (*XIIIc*)

Compound *VIIIc* (5·3 g, 17 mmol) was added to diphenyl ether (100 g) at 240°C, and the mixture was stirred at this temperature for 15 min. After cooling, hexane (500 ml) was added and the mixture was stirred at room temperature for 1 h, the separated solid was filtered off, washed with hexane and crystallized from ethanol; yield 3·1 g (69%), subl. over 220°C. For $C_{12}H_9F_2NO_4$, (269·2) calculated: 53·54% C, 3·37% H, 14·11% F, 5·20% N; found: 53·32% C, 3·53% H, 14·03% F, 4·85% N. IR spectrum: 3 550 (OH); 3 340 (NH); 1 715 (CO, COO); 1 620, 1 570, 1 520, 1 490 (arom. system). UV spectrum: λ_{max} 227 (log ε 3·40), 311 (3·07), $\lambda_{infl.}$ 320.

Ethyl 9-Nitro-3-oxo -3*H*-pyrido[3,2,1-*kl*]phenoxazin-2-carboxylate (*XIVa*)

A) A mixture of *XIIIa* (0·46 g, 2 mmol), sodium hydrogen carbonate (0·17 g, 2 mmol) and N,N-dimethylformamide (6 ml) was stirred at 100°C for 1 h, a solution of 2,4-dinitrochlorobenzene (0·42 g, 2·1 mmol) in N,N-dimethylformamide (4 ml) was added and the mixture was stirred at 100°C for 4 h. Then the reaction mixture was poured into water (30 ml), the separated solid was filtered off and crystallized from ethanol; yield 0·35 g (50%), m.p. 268·2—272·1°C. For $C_{18}H_{12}N_2O_6$ (352·3) calculated: 61·37% C, 3·43% H, 7·95% N; found: 61·69% C, 3·62% H, 8·23% N. IR spectrum: 1 720 (COO); 1 690 (CO); 1 640, 1 610, 1 590, 1 530, 1 490 (arom. system); 1 560 (NO_2); 1 280 (—O—). UV spectrum: λ_{max} 205 (log ε 3·60), 305 (3·06), 340 (2·95), 399 (3·28), $\lambda_{infl.}$ 255, 246.

B) To a solution of *XIIIa* (1·16 g, 5 mmol) and 2,4-dinitrochlorobenzene (1·01 g, 5 mmol) in ethanol (50 ml) a solution of sodium hydrogen carbonate (0·84 g, 10 mmol) in water (3, ml) was added and the mixture was refluxed for 1 h. The mixture was coooled down, the separated solid was filtered off and crystallized from ethanol; yield 1·05 g (60%), m.p. 268·4—272·2°C.

C) Sodium hydride (80% dispersion in mineral oil, 0·33 g, 11 mmol) was added to a stirred suspension of *XIIIa* (2·32 g, 10 mmol) in N,N-dimethylformamide (20 ml) and the mixture was stirred at room temperature for 1 h. Then a solution of 2,4-dinitrochlorobenzene (2·02 g, 10 mmol) in N,N-dimethylformamide (10 ml) was added, the mixture was refluxed for 4 h. The dark mixture was poured into water (200 ml), the separated tar product was filtered off, boiled in ethanol (200 ml) with charcoal (0·5 g), the filtrate was evaporated to 25 ml and cooled down. The precipitate was collected by filtration and crystallized from ethanol; yield 0·2 g (6%), m.p. 265—269°C.

9-Nitro-3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylic Acid (*XIVd*)

A) A mixture of *XIVa* (0·35 g, 1 mmol), acetic acid (3 ml), and concentrated hydrochloric acid (3 ml) was refluxed for 4 h, cooled down and the solid was filtered off and crystallized from acetic acid; yield 0·25 g (77%), not melting up to 340°C. For $C_{16}H_8N_2O_6$ (324·3) calculated: 59·27% C, 2·49% H, 8·64% N; found: 59·48% C, 2·64% H, 8·28% N. IR spectrum: 1 705 (COOH, CO); 1 630, 1 610, 1 590, 1 490 (arom. system); 1 560 (NO_2); 1 260 (—O—).

B) To a solution of *XIIIa* (1·16 g, 5 mmol) and 2,4-dinitrochlorobenzene (2·02 g, 10 mmol) in ethanol (50 ml) a solution of sodium hydrogen carbonate (1·68 g, 20 mmol) in water (25 ml)

was added and the mixture was refluxed for 8 h. The mixture was cooled down, the solid was filtered off and crystallized from acetic acid; yield 1·15 g (71%), not melting up to 340°C.

Ethyl 6-Chloro-9-nitro-3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylate (*XIVb*)

A mixture of *XIIIb* (2·68 g, 10 mmol), sodium hydrogen carbonate (0·84 g, 10 mmol), 2,4-dinitrochlorobenzene (2·02 g, 10 mmol) and N,N-dimethylformamide (50 ml) was stirred at 100°C for 8 h. The mixture was poured into water (150 ml), the separated solid was filtered off and crystallized from 80% aqueous N,N-dimethylformamide; yield 1·6 g (41%), m.p. 267·7—270·7°C. For $C_{18}H_{11}ClN_2O_6$ (386·8) calculated: 55·90% C, 2·87% H, 9·17% Cl, 7·24% N; found: 55·24% C, 2·72% H, 9·32% Cl, 7·10% N. UV spectrum: λ_{max} 205 (log ε 3·58), 304 (3·02), 331 (2·90), 390 (3·28), $\lambda_{infl.}$ 244.

6-Chloro-9-nitro-3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylic Acid (*XIVe*)

A) A mixture of *XIVb* (0·38 g, 1 mmol), acetic acid (5 ml), and concentrated hydrochloric acid (3 ml) was refluxed for 8 h, cooled down, the solid was filtered off and crystallized from N,N-dimethylformamide; yield 0·22 g (61%), not melting up to 340°C. For $C_{16}H_7ClN_2O_6$ (358·7) calculated: 53·58% C, 1·97% H, 9·88% Cl, 7·81% N; found: 53·13% C, 2·18% H, 9·49% Cl, 7·75% N. IR spectrum: 1 720 (COOH, CO); 1 620, 1 605, 1 590, 1 490 (arom. system); 1 550 (NO_2); 1 275 (—O—).

B) To a solution of *XIIIb* (2·68 g, 10 mmol) and 2,4-dinitrochlorobenzene (4·04 g, 20 mmol) in ethanol (100 ml) a solution of sodium hydrogen carbonate (3·2 g, 38 mmol) in water (50 ml) was added and the mixture was refluxed for 4 h, cooled down and the solid was filtered off and refluxed with a mixture of acetic acid (25 ml) and concentrated hydrochloric acid (25 ml) for 7 h. The precipitate was filtered off and crystallized from N,N-dimethylformamide; yield 1·4 g (39%), not melting up to 340°C.

Ethyl 5,6-Difluoro-9-nitro-3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylate (*XIVc*)

A mixture of *XIIIc* (2·69 g, 10 mmol), sodium hydrogen carbonate (1·26 g, 15 mmol) and N,N-dimethylformamide (30 ml) was stirred at 100°C for 1 h, then a solution of 2,4-dinitrochlorobenezene (2·22 g, 11 mmol) in N,N-dimethylformamide (20 ml) was added and the mixture was stirred at 100°C for 4 h. The mixture was poured into water (100 ml), the separated solid was filtered off and crystallized twice from ethanol using charcoal; yield 2·1 g (54%), m.p. 258·9 to 260·0°C. For $C_{18}H_{10}F_2N_2O_6$ (388·3) calculated: 55·68% C, 2·60% H, 9·79% F, 7·21% N; found: 55·35% C, 2·62% H, 9·75% F, 7·21% N. IR spectrum: 1 715 (COO); 1 705 (CO); 1 640, 1 610, 1 590, 1 490 (arom. system); 1 560 (NO_2); 1 260 (—O—); 1 120 (F). UV spedtrum: λ_{max} 206 (log ε 3·57), 225 (3·46), 301 (3·04), 331 (2·91), 388 (3·32), $\lambda_{infl.}$ 241.

5,6-Difluoro-9-nitro-3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylic Acid (*XIVf*)

A mixture of *XIVc* (1·94 g, 5 mmol), acetic acid (15 ml), and concentrated hydrochloric acid (15 ml) was refluxed for 4 h, cooled down, the solid was filtered off and crystallized from N,N-dimethylformamide; yield 1·6 g (88%), m.p. 331—334°C. For $C_{16}H_6F_2N_2O_6$ (360·2) calculated: 53·35% C, 1·68% H, 10·55% F, 7·78% N; found: 53·31% C, 1·93% H, 10·59% F, 8·06% N. IR spectrum: 1 705 (COOH, CO); 1 635. 1 610, 1 590, 1 480 (arom. system); 1 560 (NO_2); 1 260 (—O—); 1 120 (F). UV spectrum: λ_{max} 207 (log ε 3·06), 229 (2·95), 302 (2·40), 334 (2·37), 376 (2·78).

6-Chloro-5,9-dinitro-3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylic Acid (*XIVg*)

To a cold mixture of 98% sulfuric acid (6 ml) and 45% nitric acid (6 ml) *XIVe* was added (1 g, 2·8 mmol) and the mixture was stirred at room temperature for 10 days. The separated solid was filtered off, washed with water, dried and crystallized from N,N-dimethylformamide; yield yield 0·73 g (65%), not melting up to 340°C. For $C_{16}H_6ClN_3O_8$ (403·7) calculated: 47·60% C, 1·50% H, 8·78% Cl, 10·41% N; found: 47·88% C, 1·56% H, 8·94% Cl, 10·29% N. IR spectrum: 1 730 (COOH, CO); 1 630, 1 605, 1 590, 1 535, 1 490 (arom. system); 1 550 (NO_2); 1 270 (—O—).

5-Fluoro-6-(4-methyl-1-piperazinyl)-9-nitro-3-oxo-3*H*-pyrido-[3,2,1-*kl*]phenoxazine-2-carboxylic Acid (*XIVh*)

A mixture of *XIVf* (0·36 g, 1 mmol), N-methylpiperazine (0·4 g, 4 mmol) and pyridine (10 ml) was refluxed under nitrogen for 6 h, the mixture was evaporated to dryness under reduced pressure and boiled with ethanol (25 ml). The mixture was separated by centrifugation, the solid was treated with ethanol (25 ml), separated, treated with water (25 ml) and again separated. The solid was dried; yield 0·1 g (23%), not melting up to 340°C. For $C_{21}H_{17}F_4N_4O_6$ (440·4) calculated: 57·28% C, 3·89% H, 4·31% F, 12·72% N; found: 56·43% C, 3·86% H, 4·32% F, 12·39% N.

9-Amino-5,6-difluoro-3-oxo-3*H*-pyrido[3,2,1-*kl*]phenoxazine-2-carboxylic Acid (*XIVi*)

A suspension of *XIVf* (1·09 g, 3 mmol) in a solution of sodium hydroxide (4 g, 0·1 mol) in water (150 ml) was stirred under reflux for 0·5 h, then a solution of ferrous sulfate heptahydrate (10 g, 36 mmol) in water (50 ml) was added and the mixture was refluxed for additional 0·5 h. The hot mixture was filtered, the solid was twice boiled with water (à 200 ml), and filtered off. The aqueous solutions were acidified with acetic acid and cooled down, the precipitate was filtered off and crystallized from pyridine; yield 0·35 g (36%), not melting up to 340°C. For $C_{16}H_8F_2N_2O_4$ (330·2) calculated: 58·19% C, 2·44% H, 11·51% F, 8·48% N; found: 57·56% C, 2·84% H, 11·33% F, 8·00% N. IR spectrum: 3 260, 3 350 (NH_2); 1 720)COOH); 1 710 (CO); 1 630, 1 590, 1 510, 1 490 (arom. system); 1 255 (—O—).

9-Amino-5-fluoro-6-(4-methyl-1-piperazinyl)-3-oxo-3*H*-pyrido-[3,2,1-*kl*]phenoxazine-2-carboxylic Acid (*XIVj*)

A mixture of *XIVi* (0·33 g, 1 mmol), N-methylpiperazine (0·5 g, 5 mmol), and pyridine (20 ml) was stirred at 100°C under argon atmosphere for 20 h, the mixture was evaporated to dryness under reduced pressure. The residue was refluxed with ethanol (10 ml) for 30 min, then the mixture was cooled down, the precipitate was filtered off, washed with water and dried yielding 0·35 g of a crude product. Crystallization from pyridine yielded 0·25 g (61%); m.p. 299—307°C (decomp.). For $C_{21}H_{19}FN_4O_4$ (410·4) calculated: 61·46% C, 4·67% H, 4·63% F, 13·65% N; found: 61·34% C, 4·68% H, 4·69% F, 13·89% N. IR spectrum: 3 340, 3 250 (NH_2); 1 720 (COOH); 1 710 (CO); 1 620, 1 600, 1 520 (arom. system); 1 270 (—O—). ^{1}H NMR spectrum ($(C^2H_3)_2SO$, 140°C): 2·30 s, 3 H (NCH_3), 2·50 m, 4 H (CH_3NCH_2), 3·30 m, 4 H (CH_2), 6·50—7·50 m, 4 H (aromatic H), 8·96 s, 1 H (vinyl H). Mass spectrum, *m/z* 410 (M^+).

The Elemental analyses were carried out by Mrs J. Komancová and Dr M. Čech (Head: Dr J. Dohnal). The IR *and* UV *spectra were interpreted by Dr J. Vachek, the* ^{1}H NMR *spectra by Dr J. Holubek, and the mass spectra by Drs M. Ryska and I. Koruna.*

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