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Cinnoline Chemistry. XII. The Synthesis of 6-Fluoro-4-methylcinnoline and other Cinnolines as Potential Antitumor Agents (1)

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6-Fluoro-4-methylcinnoline (IX) has been synthesized in eight steps from 4-fluoroaniline. A series of styryl and pyridylethenyl cinnolines have been prepared from 6-fluoro-4-methylcinnoline (IX), 4-methylcinnoline and 4-methylcinnoline-2-oxide. Furthermore, a series of 4-substituted benzylthiocinnolines have been prepared.

The antitumor activity of 4-benzylthio-, 4-(2,4-dichlorobenzylthio)- and 4,6-bis(2,4-dichlorobenzylthio)cinnoline (4) provided the stimulus for the synthesis of the substituted cinnolines reported in this paper.

6-Fluoro-4-methylcinnoline was needed as an intermediate for these syntheses. To prepare it, 4-fluoroaniline (I) was allowed to react with chloral hydrate and hydroxylamine using the general procedure of Marvel and Hiers (5) as a model. The product, 4-fluoroisonitrosoacetanilide (II) was obtained in 92% yield. Compound II was converted into 5-fluoroisatin (III) in 72% yield when it was heated with sulfuric acid. 5-Fluoroisatin (III) was readily converted into the corresponding fluoroisatoic anhydride (IV) in 73% yield by allowing it to react with chromium trioxide in acetic acid-acetic anhydride solution. The fluoroisatoic anhydride (IV) was hydrolyzed in hydrochloric acid solution into 2-amino-5-fluorobenzoic acid (V) in 81% yield. The acid (V) was readily esterified with methanol in the presence of dry hydrogen chloride to give a 92% yield of methyl 2-amino-5-fluorobenzoate (VI). The ester (VI) was allowed to react with two moles of methyl magnesium iodide followed by hydrolysis and the expected 2-(2-amino-5-fluorophenyl)-2-propanol (VII) was obtained in 95% yield. Compound VII was dehydrated with phosphorus pentoxide in benzene solution to produce 2-(2-amino-5-fluorophenyl)-propene (VIII) in about 50% yield. The substituted propene (VIII) was allowed to react with sodium nitrite in acid solution, whereupon the 6-fluoro-4-methylcinnoline (IX) was obtained in 93% yield.

The condensation of 6-fluoro-4-methylcinnoline (IX) or 4-methylcinnoline (6) with a series of aldehydes gave a series of styryl-, pyridylethenyl- and furylethenylcinnolines (X-XXIV) (Table I).

4-Methylcinnoline-2-oxide (XXV) (7a-b) gave 4-(4-dimethylaminostyryl)cinnoline-2-oxide (XXVI) and 4-(4-nitrostyryl)cinnoline-2-oxide (XXVII) when allowed to react with 4-dimethylaminobenzaldehyde and 4-nitrobenzaldehyde, respectively, in the presence of potassium ethoxide. However, when benzaldehyde was allowed to react with 4-methylcinnoline-2-oxide in the presence of potassium ethoxide and an excess of benzaldehyde used as a solvent, the *N*-oxide function was lost and 4-styrylcinnoline (XXVIII) was the product. This was established by a mixed melting point determination with an authentic specimen (6). 4-Styrylcinnoline (XXVIII) was allowed to react with perphthalic acid and the product was assigned the structure 4-styrylcinnoline-2-oxide (XXIX) on the basis of the similarity of the infrared spectra of XXIX compared with those of XXVI and XXVII.

In order to prepare additional benzylthiocinnolines for antitumor screening, 6,7-dimethoxy-4-cinnolinethiol (XXX) (8), 4-cinnolinethiol (XXXI) (8) and 8-chloro-4-cinnolinethiol (XXXII) (4) were allowed to react with a series of benzyl halides. The products, XXXIII-XXXVIII are recorded in Table II.

The compounds have been screened for antitumor and tissue culture activity according to the CCNSC screening protocol (9). The following compounds exhibited statistically significant activity: 4-(4-chlorobenzylthio)-6, 7-dimethoxycinnoline (XXXV), T/C = 0.34 (SA-180); 8-chloro-4-(2,4-dichlorobenzylthio)cinnoline (XXXVIII), T/C = 0.29 (SA-180), T/C = 0.30 (CA-755). The activity of a compound is considered to be statistically significant if the T/C is 0.53 or less in SA-180 or CA-755.

EXPERIMENTAL (10)

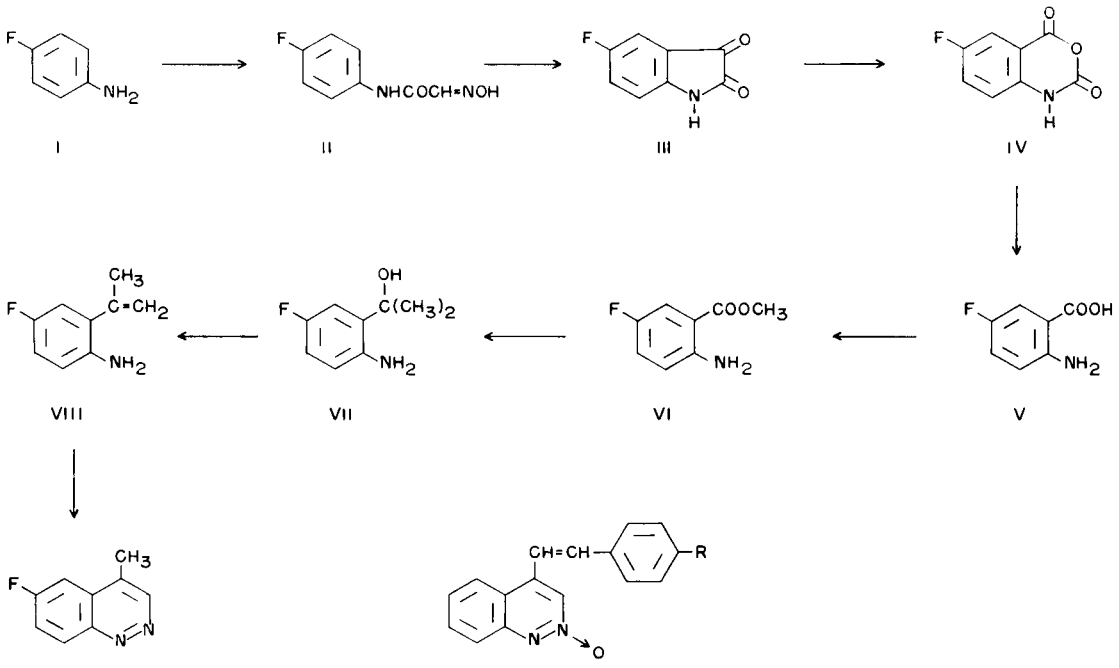
4-Fluoroisonitrosoacetanilide (II).

To a solution containing 64 g. (0.39 mole) of chloral hydrate and 486 g. of sodium sulfate in 1.5 l. of water was added a solution containing 44 g. (0.40 mole) of 4-fluoroaniline and 35 ml. of concentrated hydrochloric acid in 240 ml. of water. To this reaction mixture was added 88 g. (1.26 moles) of hydroxylamine hydrochloride in 240 ml. of water. The reaction mixture was heated vigorously with a Meker burner until refluxing began. After boiling 1-2 minutes the reaction mixture was cooled which resulted in the separation of the solid product which was filtered and dried; yield, 66 g. (92%). An analytical sample from 1:1 aqueous ethanol melted at 160°.

Anal. Calcd. for $C_8H_7FN_2O_2$: C, 52.73; N, 3.87. Found: C, 52.87; H, 3.91.

5-Fluoroisatin (III).

To 170 ml. of concentrated sulfuric acid at 70° was added 66 g. (0.36 mole) of 4-fluoroisonitrosoacetanilide (II) portionwise over a period of about 1.5 hours so that the temperature of the reaction mixture remained at 65-70°. After the addition was complete the



I II III IV V VI VII VIII XIX

XXVI, R = $N(CH_3)_2$

XXVII, R = NO_2

XXIX, R = H

reaction mixture was heated at 70-80° for an additional 10 minutes. The reaction mixture was cooled to room temperature and poured onto 2 kg. of ice. The golden yellow solid was filtered; yield 46.5 g. (72%). An analytical sample was prepared by recrystallization from either ethanol or acetic acid, m.p. 225°.

Anal. Calcd. for $C_8H_4FNO_2$: C, 58.19; H, 2.44. Found: C, 58.03; H, 2.36.

6-Fluoroisatoic Anhydride (IV).

To 5 g. (0.03 mole) of 5-fluoroisatin (III) in a mixture of 25 ml. of glacial acetic acid and 25 ml. of acetic anhydride was added 5 g. of chromium trioxide portionwise at 80-90°. During the reaction a yellow precipitate appeared. The reaction mixture was heated a few minutes, cooled, the solid was filtered and washed with water. There was obtained 4 g. of IV (73% yield). An analytical sample was obtained by recrystallization from glacial acetic acid, m.p. 270° dec.

Anal. Calcd. for $C_8H_4FNO_3$: C, 53.04; H, 2.23; N, 7.73. Found: C, 53.01; H, 1.89; N, 8.12.

2-Amino-5-fluorobenzoic Acid (V).

A reaction mixture containing 14 g. (0.077 mole) of IV in 100 ml. of concentrated hydrochloric acid-water (1:1) was heated under reflux for 2 hours, during which time there was evolution of carbon dioxide. After cooling the reaction mixture, sodium carbonate was added until the *p*H was 3. The solid which separated was filtered; yield 10.3 g. (86%). An analytical sample was recrystallized from water, m.p. 185° (pale yellow needles).

Anal. Calcd. for $C_7H_6FNO_2$: C, 54.19; H, 3.90. Found: C, 54.36; H, 3.63.

Methyl 2-amino-5-fluorobenzoate (VI).

A mixture of 30 g. (0.19 mole) of 2-amino-5-fluorobenzoic acid (V) in 150 ml. of absolute methanol was saturated with dry hydrogen chloride at ice bath temperature during a period of 30 minutes. The mixture was then heated under reflux for 4 hours during which time dry hydrogen chloride was bubbled through the mixture. After cooling, the mixture was concentrated under reduced pressure and 200 ml. of water was added to the residue which was then made alkaline with sodium carbonate. The oil that separated was extracted with ether, the ethereal solution was dried over anhydrous sodium sulfate, the ether was removed and the oil was distilled, b.p. 100-102°/0.5 mm. (30 g., 92% yield). The oil was converted to a picrate for analysis, m.p. 117°.

Anal. Calcd. for $C_{14}H_{11}FN_4O_9$: C, 42.21; H, 2.78. Found: C, 42.23; H, 2.57.

2-(2-Amino-5-fluorophenyl)-2-propanol (VII).

To the Grignard reagent prepared from 20 g. (0.82 gram atom) of magnesium, 120 g. (0.85 mole) of methyl iodide and 200 ml. of ether was added 28 g. (0.165 mole) of methyl 2-amino-5-fluorobenzoate (VI) in 100 ml. of ether during a period of 45 minutes. The reaction mixture was heated under reflux for an additional 30 minutes. After cooling the reaction mixture was poured onto ice, 50 ml. of concentrated ammonia and 300 g. of ammonium chloride. The mixture was extracted with ether, the ether solution was dried and the ether was evaporated leaving 26.5 g. of crystalline solid (95% yield) m.p. 85-90°. An analytical sample was recrystallized from cyclohexane, m.p. 92-93°.

Anal. Calcd. for $C_9H_{12}FNO$: C, 63.88; H, 7.15. Found: C, 63.72; H, 7.01.

2-(2-Amino-5-fluorophenyl)propene (VIII).

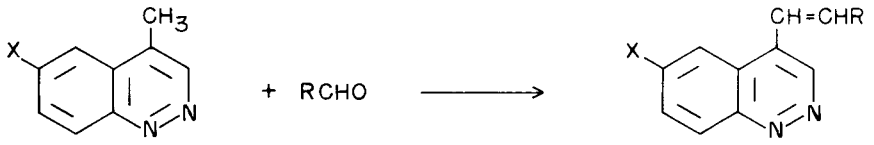
A mixture containing 26.5 g. (0.16 mole) of 2-(2-amino-5-fluorophenyl)-2-propanol (VII), 70 g. of phosphorus pentoxide and 150 ml. of benzene was heated under reflux for 3 hours. After cooling the reaction mixture, the benzene was removed by decantation and the residue was treated with 300 ml. of concentrated ammonium hydroxide and ice. The alkaline mixture was extracted with ether, dried, the ether was removed and the residue was distilled at 70-75°/0.5 mm. The yield of oil was 10-12 g. (41-50% yield). The picrate prepared for analysis recrystallized as yellow needles from benzene, m.p. 167°.

Anal. Calcd. for $C_{15}H_{13}FN_4O_7$: C, 47.37; H, 3.44. Found: C, 47.53; H, 3.49.

6-Fluoro-4-methylcinnoline (IX).

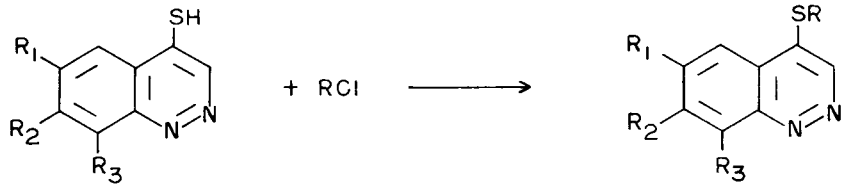
To a cold solution (2-5°) containing 10 g. (0.066 mole) of 2-(2-amino-5-fluorophenyl)propene (VIII) and 100 ml. of dilute hydrochloric acid (1:5) was added 20 ml. of concentrated hydrochloric acid (the white

TABLE I



Compound Number	R	X	Method	m.p.	Yield %	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found
X	Phenyl	F	A	180	49	Benzene	$C_{16}H_{11}FN_2$	76.78	76.76	4.43	4.12
XI	*p*-Fluorophenyl	F	A	180.5-181.5	60	Methanol	$C_{16}H_{10}F_2N_2$	71.64	71.76	3.76	3.66
XII	*m*-Fluorophenyl	F	A	175	36	Ethanol	$C_{16}H_{10}F_2N_2$	71.64	71.40	3.76	3.62
XIII	*o*-Fluorophenyl	F	A	174	72	Ethanol	$C_{16}H_{10}F_2N_2$	71.64	71.20	3.76	3.33
XIV	2-Pyridyl	F	A	155	53	Methanol	$C_{15}H_{10}FN_3$	71.69	71.61	4.01	3.95
XV	3-Pyridyl	F	A	205	53	Ethanol	$C_{15}H_{10}FN_3$	71.69	71.77	4.01	3.83
XVI	4-Pyridyl	F	B	232	26	Ethanol	$C_{15}H_{10}FN_3$	71.69	71.83	4.01	3.75
XVII	2-Pyridyl	H	A	141	50	Aqueous Ethanol	$C_{15}H_{11}N_3$	77.21	76.99	4.75	4.38
XVIII	3-Pyridyl	H	A	180	48	Aqueous Methanol	$C_{15}H_{11}N_3$	77.21	76.83	4.75	4.31
XIX	4-Pyridyl	H	A	206	33	Aqueous Ethanol	$C_{15}H_{11}N_3$	77.21	76.72	4.75	4.50
XX	*o*-Fluorophenyl	H	A	178	57	Methanol	$C_{16}H_{11}FN_2$	76.78	76.56	4.43	4.23
XXI	*m*-Fluorophenyl	H	A	168	82	Methanol	$C_{16}H_{11}FN_2$	76.78	76.67	4.43	4.29
XXII	*p*-Fluorophenyl	H	A,C	175	75	Ethanol	$C_{16}H_{11}FN_2$	76.78	76.46	4.43	3.98
XXIII	*m*-Hydroxyphenyl	H	A	250-251	51	Ethanol	$C_{16}H_{12}N_2O$	77.39	77.54	4.87	4.81
XXIV	2-Furyl	H	C	131	33	Benzene Cyclohexane	$C_{14}H_{10}N_2O$	75.66	75.40	4.54	4.31

TABLE II



Compound Number	R	R_1	R_2	R_3	m.p.°	Yield %	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
XXXIII	Benzyl	OCH_3	OCH_3	H	200	96	Ethanol	$C_{17}H_{16}N_2O_2S$	65.35	65.58	5.16	5.08	8.96	9.04
XXXIV	2-Chlorobenzyl	OCH_3	OCH_3	H	186	95	Acetic Acid	$C_{17}H_{15}ClN_2O_2S$	58.86	59.35	4.35	3.85		
XXXV	4-Chlorobenzyl	OCH_3	OCH_3	H	168	94	Ethanol	$C_{17}H_{15}ClN_2O_2S$	58.86	59.33	4.35	3.84		
XXXVI	2,4-Dichlorobenzyl	OCH_3	OCH_3	H	178-179	89	Acetic Acid	$C_{17}H_{14}Cl_2N_2O_2S$	53.54	53.53	3.70	3.51		
XXXVII	3,4-Dichlorobenzyl	OCH_3	OCH_3	H	223	96	80% Acetic Acid	$C_{17}H_{14}Cl_2N_2O_2S$	53.54	53.49	3.70	4.08		
XXXVIII	2,4-Dichlorobenzyl	H	H	Cl	213-214	48	Benzene	$C_{15}H_9Cl_3N_2S$ (a)	50.64	49.66	2.55	2.96	7.88	7.84

(a) The carbon analysis is poor even though the compound is as pure as can be obtained. Calcd.: S, 9.01; Found: S, 8.52.

hydrochloride separated). The mixture was diazotized by adding 4.8 g. of sodium nitrite in 15 ml. of water at a temperature of 2-5° over a period of 30 minutes. The reaction mixture was allowed to stand at room temperature for 1 hour then heated at 60° for 30 minutes. The reaction mixture was allowed to stand at room temperature for two days. The deep red solution was made alkaline with sodium carbonate. The pale violet crystals which separated were removed by filtration, yield 9.8 g. (93%). The purified compound recrystallized from cyclohexane, m.p. 127-128°. The picrate prepared for analysis and recrystallized from ethanol melted at 160-163° dec.

Anal. Calcd. for $C_{15}H_{10}FN_5O_7$: C, 46.04; H, 2.58. Found: C, 46.10; H, 2.64.

The Reaction of Aldehydes with 4-Methylcinnolines.

Method A.

This procedure was the zinc chloride catalyzed method described by Jacobs, *et al.*, (6) and Castle and Cox (11).

Method B.

A mixture of 1 g. (6.2 mmoles) of 6-fluoro-4-methylcinnoline, 0.5 g. (8.9 mmoles) of potassium hydroxide, 0.5 ml. of water, 20 ml. of absolute ethanol and 1.5 ml. of 4-pyridinecarboxaldehyde was heated under reflux for one hour. The ethanolic solution was reduced to one half volume under reduced pressure and the resulting precipitate was washed with water and recrystallized from ethanol, yield 0.4 g. (27% yield) of yellow needles, m.p. 232°.

Method C.

A mixture containing 2 g. (14 mmoles) of 4-methylcinnoline, 0.5 g. (13 mmoles) of potassium metal, 20 ml. of absolute ethanol and 2 ml. of furfural was heated under reflux for 5 hours. The ethanolic solution was evaporated to one half volume under reduced pressure. To the black residue was added 20 ml. of 2 *N* hydrochloric acid and 4 g. of a black hydrochloride salt was obtained. The hydrochloride was allowed to react with 30 ml. of 2 *N* sodium hydroxide and 3 g. of black free base was obtained. This was extracted continuously with 300 ml. of benzene for 3 hours. One gram (33% yield) of a brown solid was obtained after repeated crystallization from benzene or benzene-cyclohexane mixtures, m.p. 131°.

4-(4-Dimethylaminostyryl)cinnoline-2-oxide (XXVI).

To 30 ml. of absolute ethanol in which 0.24 g. (0.0061 gram atom) of potassium metal had been dissolved were added 2 g. (12 mmoles) of 4-methylcinnoline-2-oxide (7a-b) and 1.8 g. (12 mmoles) 4-dimethylaminobenzaldehyde. The brown mixture was heated for 5 hours on a steam bath, cooled and 2.15 g. of dark red crystals was obtained. The product was washed with ether. An additional amount of product was obtained from the mother liquor, total yield (crude) 2.55 g. (96% yield). The product was dissolved in 1 *N* hydrochloric acid, treated with norite and precipitated with 1 *N* sodium hydroxide, m.p. 238-241°. The analytical sample was prepared by chromatography on alumina (elution with chloroform), m.p. 247-248°.

Anal. Calcd. for $C_{18}H_{17}N_3O$: C, 74.19; H, 5.88; N, 14.42. Found: C, 74.33; H, 5.82; N, 14.32.

4-(4-Nitrostyryl)cinnoline-2-oxide (XXVII).

This compound was prepared as described for XXVI from 0.8 g. (5 mmoles) of 4-methylcinnoline-2-oxide and 0.75 g. (5 mmoles) of 4-nitrobenzaldehyde. There was obtained 0.9 g. (62% yield) of crystallized product, m.p. 287-288° from dimethyl formamide.

Anal. Calcd. for $C_{16}H_{11}N_3O_3$: C, 65.52; H, 3.78. Found: C, 65.23; H, 3.85.

The Reaction 4-Methylcinnoline-2-oxide with Benzaldehyde.

A mixture containing 1 g. of 4-methylcinnoline-2-oxide and 0.3 g. of potassium ethoxide in 20 ml. of benzaldehyde was heated under reflux for 7 hours. After the reaction mixture was allowed to stand overnight, 0.48 g. of a white nitrogen-free solid, m.p. >360° was separated and washed with ether. This product was not investigated further. The benzaldehyde-ether solution was shaken with 1 *N* hydrochloric acid with warming. Upon cooling a yellow solid separated which was filtered, washed with cold 1 *N* hydrochloric acid, then with ether and dried. The solid amounted to 0.77 g. The free base was obtained by treatment with 1 *N* sodium hydroxide solution. The product separated as fine yellow needles upon recrystallization from aqueous ethanol, m.p. 127-128°. A mixed melting point with 4-styrylcinnoline (6) showed no depression, 126-128°. Therefore the oxygen function was removed during the reaction presumably by the excess benzaldehyde.

4-Styrylcinnoline-2-oxide (XXIX).

A mixture of 4.5 g. (19 mmoles) of 4-styrylcinnoline (6) and monoperphthalic acid (from 18.5 g. of phthalic acid and 15.8 ml. of 30% hydrogen peroxide) in 200 ml. of ether was allowed to stand at room temperature for 4 days. The ether solution was then allowed to evaporate to 10-15 ml. in air, 200 ml. of water was added and the mixture was made alkaline with sodium carbonate. The yellow solid was filtered, washed with water and dried (3.5 g.). After recrystallization from aqueous ethanol, the product melted over a range (182-215°). After chromatography on alumina (elution with chloroform) and extraction of the residue with boiling ethyl acetate, the soluble portion separated and melted at 201-203°. After recrystallization from ethanol, yellow needles were obtained, m.p. 205-207°. The structure was assigned from the infrared spectrum.

Anal. Calcd. for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.29. Found: C, 77.50; H, 5.25; N, 11.33.

4-Benzylthiocinnolines (Table II).

These compounds were prepared as described by Castle, Shoup, Adachi and Aldous (4).

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(10) All melting points are uncorrected.

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