NITROGEN-CONTAINING CARCINOGENIC COMPOUNDS LXXXVII

SYNTHESIS OF FLUORINATED AND TRIFLUOROMETHYLATED INDOLO[2,3-a]CARBAZOLES AND INDOLO [2,3-a]ACRIDINES [1].

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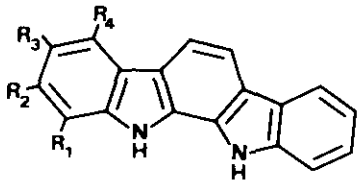
Abstract - Within the general context of our research linked to mutagenesis and chemical carcinogenesis, compounds related to certain indolo[2,3-a]carbazoles and indolo[2,3-a]-acridines bearing a fluorine atom or a trifluoromethyl group are described.

Within the general context of our research related to mutagenesis and to chemical carcinogenesis, we have described several molecules belonging to the families of the indolo[2,3-a]carbazoles and indolo[2,3-a]acridines originating from various carbazolones (1-keto-1,2,3,4-tetrahydrocarbazoles)-containing a fluorine atom or a trifluoromethyl group.

This orientation follows along from experiments carried out using series of thiopyrano[4,3-b]indoles and thiopyrano[4,3-b]quinoleines substituted in an identical manner and with various mutagenic and carcinogenic properties related to slight alterations in structure.

A few years ago, we synthesized fluoro and trifluoromethyl thiopyrano[4,3-b]indoles and thiopyrano [4,3-b]quinoleines [2,3]. The biological results obtained in the mouse (strain XVII NC/Z) indicated a major difference in activity according to whether the fluorine or trifluoromethyl substituants were fixed to one or other summit of the molecule [4]. There follow a number of examples : amongst the fluorinated thiopyrano indoles, 2-fluoro-6,13-dihydro-(1)benzothiopyrano[4,3-b]benzo(e)indole gives 0% of tumors whilst its isomer fluorinated in position 4 causes 93 % ; similarly, amongst trifluoromethylated molecules, 3-trifluoromethyl-6,13-dihydro-(1)benzo(e)thiopyrano[4,3-b]indole does not cause any tumors whilst its isomer substituted in position 4 gives 15 %. Similar observations may be made in the group of 7-methyl-6H(1)-benzothiopyrano[4,3-b]quinoleines, where the difference in activity between molecules fluorinated respectively in position 3 or 4 is 85 %. Furthermore, on many occasions it has been shown that benzacridines and benzocarbazoles possess properties which are definite from the standpoint of chemical carcinogenesis, but which nevertheless vary in relation to the nature and number of the substituants [5,6]. These various reasons justify

the preparation of substances containing on the one hand the indolecarbazole double sequence and, secondly, the association indoleacridine, all containing the groups mentioned above.



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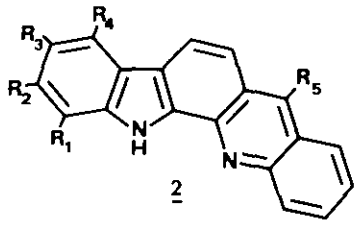

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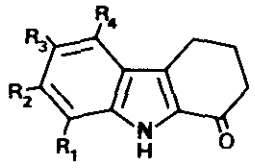

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The basic nuclei of the different indolocarbazole isomers have already been prepared and studied [7,8,9]. This is the case in particular for indolo[2,3-a]carbazole (1) [10] but few derivatives are known.

With regard to indoloacridines of general formula (2), very few syntheses have been carried out [11] and this applies even more to the substituants which are of interest to us here.

The common raw materials used to produce the various compounds were carbazolones (1-keto-1,2,3,4-tetrahydrocarbazoles). The basic structure (3) is known but no fluorinated of trifluoromethylated derivative has been described up to the present time.

The following carbazolones have been isolated :

1-keto-1,2,3,4-tetrahydrocarbazole (3) already prepared by Kent [12], amongst others.

5-fluoro-1-keto-1,2,3,4-tetrahydrocarbazole (3a) : $R^1 = R^2 = R^3 = H$; $R^4 = F$

6-fluoro-1-keto-1,2,3,4-tetrahydrocarbazole (3b) : $R^1 = R^2 = R^4 = H$; $R^3 = F$

8-fluoro-1-keto-1,2,3,4-tetrahydrocarbazole (3c) : $R^2 = R^3 = R^4 = H$; $R^1 = F$

5-trifluoromethyl-1-keto-1,2,3,4-tetrahydrocarbazole (3d) : $R^1 = R^2 = R^3 = H$; $R^4 = CF_3$

7-trifluoromethyl-1-keto-1,2,3,4-tetrahydrocarbazole (3e) : $R^1 = R^3 = R^4 = H$; $R^2 = CF_3$

8-trifluoromethyl-1-keto-1,2,3,4-tetrahydrocarbazole (3f) : $R^2 = R^3 = R^4 = H$; $R^1 = CF_3$

Application of the indolization reaction according to Fischer to these molecules enabled us to isolate indolo[2,3-a]carbazoles, though we were unable to stop at the stage of the dihydrogenated intermediate substance.

In this way, we obtained respectively from 3a, 3b, 3d, 3e and 3f the following indolo[2,3-a]

carbazoles :

7-fluoro-indolo[2,3-a]carbazole (1a) : $R^1 = R^2 = R^3 = H$; $R^4 = F$

8-fluoro-indolo[2,3-a]carbazole (1b) : $R^1 = R^2 = R^4 = H$; $R^3 = F$

7-trifluoromethyl-indolo[2,3-a]carbazole (1c) : $R^1 = R^2 = R^3 = H$; $R^4 = CF_3$

9-trifluoromethyl-indolo[2,3-a]carbazole (1d) : $R^1 = R^3 = R^4 = H$; $R^2 = CF^3$

10-trifluoromethyl-indolo[2,3-a]carbazole (1e) : $R^2 = R^3 = R^4 = H$; $R^1 = CF_3$

In the case of indoloacridine, there were two possibilities : either the preparation of molecules non-substituted in the para position of acridine nitrogen, or, again, substances containing a methyl group on this same summit. Numerous previous biological studies have demonstrated the importance of such a substitution [13].

With regard to the first category, the raw materials were condensed with orthoaminobenzaldehyde hydrochloride. For the second, orthoaminoacetophenone hydrochloride was used.

The following indoloacridines were isolated from these various raw materials :

7-methyl-indolo[2,3-a]acridine (2a) : $R^1 = R^2 = R^3 = R^4 = H$; $R^5 = CH^3$ from 3

4-fluoro-indolo[2,3-a]acridine (2b) : $R^1 = R^2 = R^3 = R^5 = H$; $R^4 = F$ and

4-fluoro-7-methyl-indolo[2,3-a]acridine (2c) : $R^1 = R^2 = R^3 = H$; $R^4 = F$; $R^5 = CH_3$ from 3a

3-fluoro-indolo[2,3-a]acridine (2d) : $R^1 = R^2 = R^4 = R^5 = H$; $R^3 = F$ and

3-fluoro-7-methyl-indolo[2,3-a]acridine (2e) : $R^1 = R^2 = R^4 : H$; $R^3 = F$; $R^5 = CH_3$ from 3b

1-fluoro-indolo[2,3-a]acridine (2f) : $R^2 = R^3 = R^4 = R^5 = H$; $R^1 = F$ and

1-fluoro-7-methyl-indolo[2,3-a]acridine (2g) : $R^2 = R^3 = R^4 = H$; $R^1 = F$; $R^5 = CH_3$ both from 3c

1-trifluoromethyl-indolo[2,3-a]acridine (2h) : $R^2 = R^3 = R^4 = R^5 = H$; $R^1 = CF_3$ and

1-trifluoromethyl-7-methyl-indolo[2,3-a]acridine (2i) : $R^2 = R^3 = R^4 = H$; $R^1 = CF_3$; $R^5 = CH_3$ from 3f.

EXPERIMENTAL PART

Preparation of carbazolones

1-Keto-1,2,3,4-tetrahydrocarbazole (3) : according to the method of Kent, 10g of cyclohexane 1,2-dione monophenylhydrazone is dissolved in 60ml of concentrated hydrochloric acid and after 2 minutes boiling, the mixture is diluted with 40ml of water. It is filtered and recrystallized (acetic acid) small yellow needles mp = 169° (Litt. mp = 169°) [12].

5-Fluoro-1-keto-1,2,3,4-tetrahydrocarbazole (3a) : 2,7g (0.02 Mol) of 2-hydroxymethylene-cyclohexanone are dissolved in 15ml of methanol. A concentrated aqueous solution of 5g of sodium acetate is added. To this mixture, a solution containing 0.02 mol of the diazonium salt of 3-fluoro-aniline dissolved in hydrochloric acid solution is added dropwise, being kept at 0°. This gives a

syrupy substance which does not solidify and is extracted in chloroform. This organic phase is washed with a sodium carbonate solution, then water and dried on sodium sulfate. After filtration, the solvent is eliminated and raw arylhydrazone recovered. Mention should be made of the technique of the preparation of arylhydrazone according to Shvedov [15]. This intermediary substance is heated for a few minuts in acetic acid saturated with hydrogen chloride. It is allowed to cool, and poured into ammoniacal water, dried, washed abundantly in water, then recrystallized. Yield 26 %, mp = 202-203°, small colourless needles (cyclohexane-ethylacetate).

Anal. Calculated for $C_{12}H_{10}NOF$: C, 70.9 ; H, 5.0 ; N, 6.9

Found : C, 70.6 ; H, 5.3 ; N, 6.7

NMR on single proton : 7.5 shows an ortho coupling H-F i.e. $J_{6,F}$= 9,7 Hz which tends to prove that one single position is free in the ortho of fluorine, thus confirming the proposed structure (Varian T60 ; $CDCl_3$).

6-Fluoro-1-keto-1,2,3,4-tetrahydrocarbazole (3b) : proceed as for 3a but starting with 4-fluoro-aniline. Yield 72 %, mp = 211-212°, lemon yellow microcrystals (ethanol).

Anal. Calculated for $C_{12}H_{10}NOF$: C, 70.9 ; H, 5.0 ; N, 6.9

Found : C, 70.7 ; H, 5.4 ; N, 6.8

8-Fluoro-1-keto-1,2,3,4-tetrahydrocarbazole (3c) : same technique as for 3b, but starting with 2-fluoroaniline. Yield 10 %, mp = 119°, colourless microneedles (ligroin).

Anal. Calculated for $C_{12}H_{10}NOF$: C, 70.9 ; H, 5.0 ; N, 6.9

Found : C, 70.6 ; H, 5.2 ; N, 6.7

5-Trifluoromethyl-1-keto-1,2,3,4-tetrahydrocarbazole (3d) : same technique as for 3c but beginning with 3-trifluoromethylaniline. Yield 40 %, mp = 166°, colourless microcrystals (ligroin).

Anal. Calculated for $C_{13}H_{10}NOF_3$: C, 61.7 ; H, 4.0 ; N, 5.5

Found : C, 61.6 ; H, 4.1 ; N, 5.7

7-Trifluoromethyl-1-keto-1,2,3,4-tetrahydrocarbazole (3e). forms at same time as 3d. Yield 45 % mp = 252-254°, colourless microprisms (ethanol).

Anal. Calculated for $C_{13}H_{10}NOF_3$: C, 61.7 ; H, 4.0 ; N, 5.5

Found : C, 61.6 ; H, 4.1 ; N, 5.7.

The two carbazolones 3d and 3e are separated by silicagel chromatography. The structure of 3e is established as follows : the aromatic part of the NMR spectrum has a narrow signal at 7.93 (H_8) with a meta coupling of the order of 2Hz ($J_{6,8}$) ; a double bond at 7.74 (H_5) (ortho coupling 8Hz : $J_{5,6}$) and a double doublet at 7.41 (H_6) (ortho coupling 8Hz : $J_{6,5}$ and meta coupling of 2Hz : $J_{6,8}$) ; measurements on Varian A 100, Pyridine D_5.

8-Trifluoromethyl-1-keto-1,2,3,4-tetrahydrocarbazole (3f) :

obtained as 3a but from ortho trifluoromethylaniline.

Yield 28 %, mp = 130°, colourless microcrystals (ligroin)

Anal. Calculated for $C_{13}H_{10}NOF_3$: C, 61.6 ; H, 4.0 ; N, 5.5.

Found : C, 61.6 ; H, 4.4 ; N, 5.7.

Preparation of indolo[2,3-a]carbazoles

One-hundredth of a mol of carbazolone and rectified phenylhydrazine (+ 10 %) are mixed in the presence few drops of acetic acid and heated for an hour on a hot plate at 170-180° approximately. Water is eliminated and, after cooling, a coarse precipitate of arylhydrazone is formed which is cyclized in that form. It is dissolved in 40ml of acetic acid saturated with hydrogen chloride and heated at boiling point until a precipitate develops (ammonium chloride). It is allowed to cool, poured into aquous ammonia and extracted according to the individual case, in either chloroform or ether. The organic layer is washed with water, then dried on calcium chloride.

After filtration, the solvant is eliminated and a gummy, brown residue obtained. This is subsequently purified by silicagel chromatography using benzene as an eluant. The eluate is evaporated and indolo[2,3-a]carbazole recrystallized in the appropriate solvent.

The structures of carbazolones being demonstrated either by determination of the position of the substituent on the initial aromatic amine, or by the factors described above, there is no ambiguity with regard to the identity of the indolocarbazoles and indoloacridines.

7-Fluoro-indolo[2,3-a]carbazole (1a) : mp : 360-364°, colourless microcrystals (xylene) obtained from 3a.

Anal. Calculated for $C_{18}H_{11}N_2F$: C, 78.8 ; H, 4.0 ; N, 10.2.

Found : C, 78.7 ; H, 4.0 ; N, 10.0

8-Fluoro-indolo[2,3-a]carbazole (1b) isolated from 3b : mp = 365°, silvery microcrystals (xylene).

Anal. Calculated for $C_{18}H_{11}N_2F$: C, 78.8 ; H, 4.0 ; N, 10.2.

Found : C, 78.7 ; H, 4.4 ; N, 10.2.

7-Trifluoromethyl-indolo[2,3-a]carbazole (1c) obtained from 3d : mp = 345°, yellow microcrystals (xylene).

Anal. Calculated for $C_{19}H_{11}N_2F_3$: C, 70.3 ; H, 3.4 ; N, 8.6.

Found : C, 70.5 ; H, 3.6 ; N, 8.8.

9-Trifluoromethyl-indolo[2,3-a]carbazole (1d) obtained from 3e : mp = 346°, dark reddish-yellow microcrystals (xylene).

Anal. Calculated for $C_{19}H_{11}N_2F_3$: C, 70.3 ; H, 3.4 ; N, 8.6.

Found : C, 70.6 ; H, 3.6 ; N, 8.6.

10-Trifluoromethyl-indolo[2,3-a]carbazole (1e) obtained from 3f : mp = 204°, colourless straws (hexane).

Anal. Calculated for $C_{19}H_{11}N_2F_3$: C, 70.3 ; H, 3.4 ; N, 8.6.
Found : C, 70.5 ; H, 3.6 ; N, 8.8.

In all cases, yields in relation to carbazolones varied between 5 and 15 %. 5,6-Dihydro-indolo [2,3-a]carbazoles were never isolated. Each indolo[2,3-a]carbazole gave a colour ranging from pink to violet by heating in an acetic medium with tetrachlorophtalic anhydride [16].

Preparation of indolo[2,3-a]acridines.

A mixture of 15 mmol of carbazolone and 10 mmol of freshly prepared orthoaminobenzaldehyde are heated for 45 minutes at 140° (for non-methylated indolo[2,3-a]acridines) or orthoaminoacetophenone hydrochloride (for methylated indolo[2,3-a]acridines). After cooling, the substance is decomposed by diluted ammonia and extracted in chloroform. The residue obtained by evaporation of the organic phase is treated with a solution of picric acid. After washing with alcohol the picrate is broken down and the raw indolo[2,3-a]acridine is subjected to chromatography on a silicagel column, using, as eluant, benzene and then a mixture of benzene and alcohol. In the majority of cases spontaneous dehydrogenation takes place. On the few rare occasions when the 5,6-dihydro derivative was detected, it was easily dehydrogenated by heating with 5 % palladium charcoal.

4-Fluoro-indolo[2,3-a]acridine (2b) : mp = 230°, dark reddish yellow microcrystals (ethanol).

Anal. Calculated for $C_{19}H_{11}N_2F$: C, 79.7 ; H, 3.9 ; N, 9.8.
Found : C, 79.5 ; H, 4.1 ; N, 9.5.

Its picrate : mp = 288°, carmine red microcrystals (xylene).

Anal. Calculated for $C_{25}H_{14}N_5O_7$: N, 13.5 ; Found : N, 13.5.

3-Fluoro-indolo[2,3-a]acridine (2d) : mp = 220°, yellow straws (ethanol).

Anal. Calculated for $C_{19}H_{11}N_2F$: C, 79.7 ; H, 3.9 ; N, 9.8.
Found : C, 79.8 ; H, 3.8 ; N, 9.4.

Its picrate : mp = 290°, brick red microcrystals (ethanol).

Anal. Calculated for $C_{25}H_{14}N_5O_7F$: N, 13.5. Found : N, 13.5.

1-Fluoro-indolo[2,3-a]acridine (2f) : mp = 212°, small yellow needles (ethanol).

Anal. Calculated for $C_{19}H_{11}N_2F$: C, 79.7 ; H, 3.9 ; N, 9.8.
Found : C, 79.5 ; H, 3.8 ; N, 9.6.

Its picrate : mp = 286°, dark red microcrystals (xylene).

Anal Calculated for $C_{25}H_{14}N_5O_7F$: N, 13.5. Found : N, 13.4.

1-Trifluoromethyl-indolo[2,3-a]acridine (2h) : mp = 202°, pale yellow microcrystals (cyclohexane).

Anal. Calculated for $C_{20}H_{11}N_2F_3$: C, 71.4 ; H, 3.3 ; N, 8.3.

Found : C, 71.4 ; H, 3.5 ; N, 8.3.

These four indolo[2,3-a]acridines were prepared by respectively condensing 3a, 3b, 3c and 3f with orthoaminobenzaldehyde hydrochloride.

7-Methyl-indolo[2,3-a]acridine (2a) : mp = 240°, orange-yellow microcrystals (ethanol).

Anal. Calculated for $C_{20}H_{14}N_2$: C, 85.2 ; H, 5.0 ; N, 9.9.

Found : C, 85.1 ; H, 5.2 ; N, 9.8.

Its picrate : mp = 280°, brick red microcrystals (xylene).

Anal. Calculated for $C_{26}H_{17}N_5O_7$: N, 13.7. Found : N, 13.7.

4-Fluoro-7-methyl-indolo[2,3-a]acridine (2c) : mp = 254°, orange microcrystals (cyclohexane).

Anal. Calculated for $C_{20}H_{13}N_2F$: C, 80.0 ; H, 4.4 ; N, 9.3.

Found : C, 80.1 ; H, 4.4 ; N, 9.5.

Its picrate : mp = 297°, brick red microcrystals (xylene).

Anal. Calculated for $C_{26}H_{16}N_5O_7F$: N, 13.2. Found : N, 13.1.

3-Fluoro-7-methyl-indolo[2,3-a]acridine (2e) : mp = 242°, dark yellow straws (ethanol).

Anal. Calculated for $C_{20}H_{13}N_2F$: C, 80.0 ; H, 4.4 ; N, 9.3.

Found : C, 79.8 ; H, 4.4 ; N, 9.1.

Its picrate : mp = 312°, red microcrystals (chlorobenzene).

Anal. Calculated for $C_{46}H_{31}N_7O_7F$: (hemipicrate) : N, 11.8.

Found : N, 11.8.

1-Fluoro-7-methyl-indolo[2,3-a]acridine (2g) : mp = 188°, orange straws (ethanol).

Anal. Calculated for $C_{20}H_{13}N_2F$: C, 80.0 ; H, 4.4 ; N, 9.3.

Found : C, 79.8 ; H, 4.6 ; N, 9.1.

Its picrate : mp = 292°, red microcrystals (xylene).

Anal. Calculated for $C_{26}H_{16}N_5O_7F$: N, 13.2. Found : N, 13.2.

1-Trifluoromethyl-7-methyl-indolo[2,3-a]acridine (2i) :

mp = 207°, pale yellow needles (ethanol).

Anal. Calculated for $C_{21}H_{13}N_2F_3$: C, 72.0 ; H, 3.7 ; N, 8.0.

Found : C, 71.9 ; H, 3.7 ; N, 7.9.

Its picrate : mp = 306°, orange needles (ethanol).

Anal. Calculated for $C_{27}H_{16}N_5O_7F_3$: N, 12.0. Found : N, 11.9.

This five methylated indolo[2,3-a]acridines were prepared by orthoamino acetophenone hydrochloride respectively with 3, 3a, 3b, 3c and 3f. Mean yields from these syntheses of indoloacridines was 15 %.

The results of biological investigation of the various compounds will be reported later.

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