[CONTRIBUTION FROM THE LABORATORY OF RADIOCHEMISTRY, UNIVERSITY OF CINCINNATI]

COMPOUNDS FOR CANCER RESEARCH. II.[1] FLUORENE-2-CARBOXAMIDINE

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Several compounds containing the amidino grouping (—C:NH·NH_2) have been found to have therapeutic properties. For example, in 1938 Hughes, Lourie, and Yorke (1) found that 1,11-undecanedicarboxamidine had a definite action on human simple tertian malaria, causing the parasites to disappear from the peripheral blood and the febrile paroxysms to cease. Further investigations (2) showed that this and other amidines had a powerful trypanocidal action *in vitro*. Aromatic amidines showed a similar effect (3).

Andrews, King, Van den Ende, and Walker (4) found that *p*-sulfamidobenzamidine (V 147) was much more effective against infections of rickettsia murine and epidemic typhus in mice than was the ester, methyl *p*-sulfonylbenzamidine (V 187). Unfortunately no therapeutic benefit was observed in man. The latter compound, however, proved outstanding in its activity against gas gangrene and haemolytic streptococci.

The amidino group may also be considered to be combined with sulfur in thiourea or with an imino group in guanidine (5). Derivatives of each type have been shown to raise the blood pressure, to increase pulmonary ventilation, and enhance response to adrenalin.

In 1938 Blaschko (6) reported that the enzymatic oxidation of diamines such as putrescine or histamine by extracts of pig's kidney was somewhat inhibited by guanidine. Zeller (7) found that synthalin (decamethylenediguanidine) was a more powerful inhibitor.

In a study of the inhibition of amine oxidase by straight chain amidines Blaschko and Duthie (8) observed the maximum effect when x = 12 in CH_3-$(CH_2)_x$C:NH·NH_2. It will be noted that the optimum has a total of 14 carbon atoms. This fact will be referred to later.

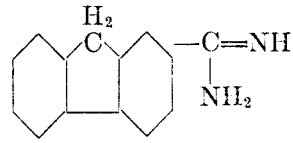
Stilbamidine, 4-NH_2·NH:CC_6H_4CH:CHC_6H_4C:NH·NH_2-4′, has been used with considerable success in the treatment of kala azar, an Indian disease that is characterized by an increase in the globulin content of the serum. Hartwell (12) was led to examine the effects of this compound on tumor bearing mice. He reported that "stilbamidine was active in damaging tumor cells of intramuscular implants of sarcoma 37." In clinical trials Snapper (13) found that "injections of stilbamidine have a favorable influence upon the pains of many patients with multiple myeloma .... who followed a diet low in protein." In certain types of tissue culture Kopac (14) states that stilbamidine selectively destroyed neoplastic cells from mammary adenocarcinoma R2426 (rat) and transplantable lymphosarcoma (rat).

[1] Part I. Organic Radioiodo Compounds for Cancer Research, Bloch and Ray, *J. Natl. Cancer Inst.*, **7**, 61 (1946).

It seemed profitable, therefore, to join the amidino group with other moieties. To be most effective this group should be combined with a structure that has a known relationship to the cancer cell. Such substances are the carcinogenic hydrocarbons, butter yellow and 2-acetylaminofluorene.

We selected the last named because of its properties of causing cancer in a variety of tissues and at points distant from the site of application, which make it closer in its relationship to natural cancer (9).

It will be seen that fluorene-2-carboxamidine contains 14 carbon atoms; and it will be recalled that Blaschko and Duthie found 14 carbons to give the maximum effect in the aliphatic series (8).



Fluorene–2–carboxamidine

Fluorene was the starting material. It was nitrated, reduced to the amine, diazotized, converted to the cyano derivative, and finally to the amidine. The details will be found in the Experimental Procedure.

Fluorene-2-carboxamidine hydrochloride was obtained in long, light yellow needles from alcohol. It melted at 290° with decomposition and was quite soluble in water but insoluble in ether and benzenes.

The free base was also soluble in alcohol from which light yellow crystals melting at 190° were obtained. It was not appreciably soluble in water but was soluble in organic solvents.

ACKNOWLEDGMENT

Our thanks are due to Dr. C. P. Rhoads for a grant from the Sloan-Kettering Institute for Cancer Research which made this work possible. Both 2-cyanofluorene and fluorene-2-carboxamidine hydrochloride are now under test at the Institute and the results will be reported in a subsequent publication.

EXPERIMENTAL PROCEDURE

2-Nitrofluorene and 2-aminofluorene were prepared according to the procedure in Organic Syntheses (10).

2-Cyanofluorene was prepared by a modification of Fortner's method (11). Dry hydrogen chloride was passed into a solution of 18.1 g. (0.1 *M*) of 2-aminofluorene in 100 cc. of benzene until no further precipitation of 2-aminofluorene hydrochloride occurred. This was filtered and suspended in 25 cc. of concentrated hydrochloric acid, cooled to 0°, and diazotized by adding 6.9 g. of sodium nitrite in a saturated solution until nitrous acid persisted for 10 minutes (starch-iodide paper test). Fluorene-2-diazonium chloride precipitated, was filtered and washed, first with alcohol and finally with ether.

It was suspended in 35 cc. of water and added in small portions to a refluxing solution of 24 g. (0.37 *M*) of potassium cyanide and 22 g. (0.14 *M*) of copper sulfate in 100 cc. of water. After boiling for 1.5 hours, nitrogen evolution ceased and the brown solid was removed by filtration. When dry it was extracted with four 75-cc. portions of ethanol. The alcoholic solution was boiled with 1 g. of charcoal, filtered, and treated with an equal volume of water to precipitate the partially purified 2-cyanofluorene. It now melted at 78°.

After 3 recrystallizations from petroleum ether of boiling point 70–90°, light yellow crystals melting at 92° were obtained. This agrees, substantially, with Fortner's value of 88°.

Anal. Calc'd for $C_{14}H_{11}N$: N, 7.33. Found: N, 7.5, 7.55.

Sometimes when recrystallization was carried out very slowly the melting point obtained was 105°, and the compound was in the form of long, light yellow needles. This is not uncommon in the fluorene series. The analysis and the subsequent reactions showed it to be the same compound, probably a polymorphic crystalline form.

Anal. Calc'd for $C_{14}H_{11}N$: N, 7.33. Found: N, 7.40, 7.36.

The high-melting form of 2-cyanofluorene has not been reported previously.

Fluorene-2-carboxamidine. Dry hydrogen chloride was passed into a suspension of 9.5 g. (0.05 *M*) of 2-cyanofluorene in 500 cc. of absolute alcohol. Heat was evolved and the solid slowly went into solution. The reaction was maintained at room temperatures by cooling if necessary. A purple solution resulted. After standing for two days, ethyl fluorene-2-imidate hydrochloride had crystallized out. It melted at 135°. A further amount was obtained by evaporating the mother liquor in a current of air.

The solid imino ester was dissolved in 85 cc. of 10% alcoholic ammonia. After 18 hours, fluorene-2-carboxamidine hydrochloride had crystallized out. It melts with decomposition at 290° on the copper block. It was soluble in hot alcohol, sparingly soluble in cold alcohol, but soluble in cold water.

Anal. Calc'd for $C_{14}H_{13}ClN_2$: N, 11.4. Found: N, 11.4, 11.6.

The free base was obtained from the hydrochloride by dissolving the latter in water, filtering if not clear, and adding an excess of 10% sodium hydroxide solution. The fluorene-2-carboxamidine precipitated and was recrystallized from alcohol. It melted at 190° with decomposition. It was not soluble in water but was soluble in organic solvents.

Anal. Calc'd for $C_{14}H_{12}N_2$: N, 13.5. Found: N, 13.3, 13.2.

SUMMARY

The possible therapeutic value of combining the amidino group and the fluorene molecule is discussed and the synthesis of fluorene-2-carboxamidine is described in detail. The results of its use in cancer studies will be reported later.

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REFERENCES

(1) HUGHES, LOURIE, AND YORKE, *Ann. Trop. Med.*, **32**, 103 (1938).
(2) KING, LOURIE, AND YORKE, *Ann. Trop. Med.*, **32**, 177 (1938).
(3) ASHLEY, *J. Chem. Soc.*, 106 (1942). *Cf.* ASHLEY AND HARRIS, *J. Chem. Soc.*, 567 (1946).
(4) ANDREWS, KING, VAN DEN ENDE, AND WALKER, *Lancet*, **1**, 777 (1944).
(5) FASTUR, *Nature*, **154**, 426 (1944).
(6) BLASCHKO, 16th. Internat. Physiol. Kongress Zurich, Kongressbericht, **3**, 73 (1938); *J. Physiol.*, **95**, 30P (1939).
(7) ZELLER, *Helv. Chim. Acta*, **21**, 717 (1938).
(8) BLASCHKO AND DUTHIE, *Biochem. J.*, **39**, 347 (1945).
(9) ARMSTRONG AND BONSER, *J. Path. Bact.*, **59**, 19 (1947).
HARRIS, *Cancer Res.*, **7**, 88 (1947).
WESTFALL, *J. Natl. Cancer Inst.*, **6**, 23 (1946).
(10) *Org. Syntheses*, **13**, 74 (1933). *Cf.* SAMPEY AND REID, *J. Am. Chem. Soc.*, **69**, 7112 (1947).
(11) FORTNER, *Monatsh.*, **25**, 443 (1904).
(12) HARTWELL, *Cancer Res.*, **6**, 489 (1946).
(13) SNAPPER, *J. Mt. Sinai Hosp.*, **13**, 119 (1946).
(14) KOPAC, *Cancer Res.*, **6**, 491 (1946).