

COMPOUNDS FOR CANCER RESEARCH. IV. FURTHER  
BIFLUORYL AND BIFLUORYLIDENE DERIVATIVES<sup>1</sup>

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Pinck's (1) recent hypothesis of the genesis of cancer attempts to explain the production of tumors by certain chemical agents according to the following mechanism. The carcinogenic compound, which must contain active hydrogens, is dehydrogenated *in vivo* to an ethylenic dimer. Cell substance, containing at least one active hydrogen, condenses with the ethylene so formed. Thus a chain of reactions is initiated which leads to the formation of a very large molecule of cell substance—potential cancer tissue.

Pinck concluded that the carcinogenic activity of 2-acetylaminofluorene (I) is due to the 2,2'-diacetyl-amino-9,9'-bifluorylidene (III) formed from I. Two reactions leading to the formation of the carcinogen were postulated.

A necessary, although not sufficient condition, if the hypothesis is to hold, is to establish the carcinogenic character of the compounds in question, both 2,2'-diacetyl-amino-9,9'-bifluoryl (II) and the bifluorylidene, III.

This hypothesis may explain the mode of action of 2-acetylaminofluorene in causing cancer but gives no explanation for the production of tumors in organs distant from the site of application. Possibly, when the 2-acetylaminofluorene enters the body, part is hydrolyzed to 2-aminofluorene which is solubilized by the purines present in the blood stream (2). It can thus be carried about the body to various organs where tumors form because of the reaction postulated by Pinck.

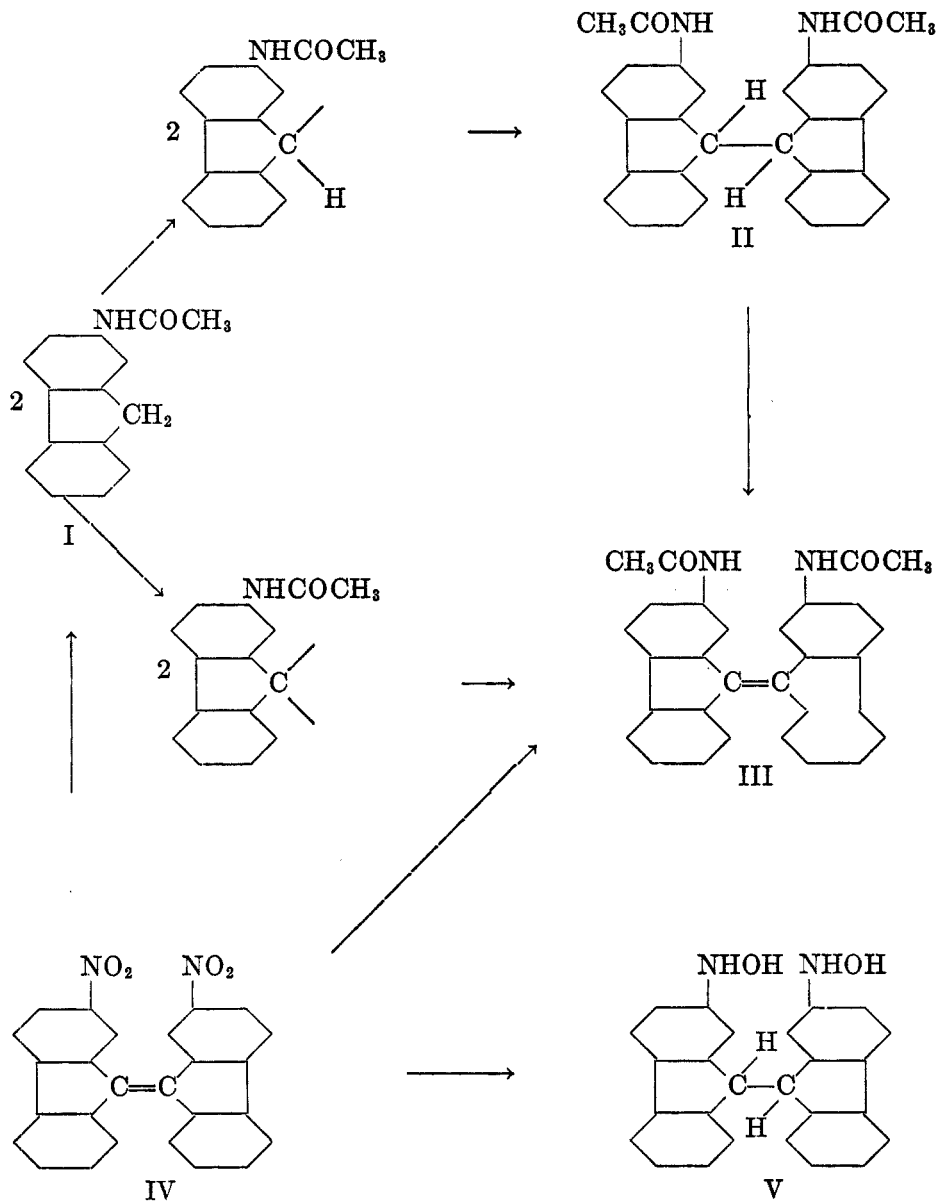
In order to obtain experimental evidence concerning Pinck's hypothesis 2,2'-diacetyl-amino-9,9'-bifluoryl and bifluorylidene were prepared. It has been shown that 2-nitro-9-iodofluorene does not spontaneously lose iodine with formation of 2,2'-dinitro-9,9'-bifluoryl (3). However, the corresponding bifluorylidene derivative was readily prepared by dehydrohalogenation of 2-nitro-9-bromofluorene with potassium hydroxide in methanol-acetone solution according to the method of Thiele and Wanscheidt (4).

The dinitro derivative, IV, was reduced to the intermediate 2,2'-dihydroxyl-amino-9,9'-bifluoryl (V), with zinc and calcium chloride in an ethanol-dioxane solution. Reduction of either IV or V by zinc dust and acetic acid yielded 2,2'-diacetyl-amino-9,9'-bifluoryl (II). No attempt was made to separate or identify any *meso* or *racemic* forms.

The authors observed on previous occasions that at the melting point a bifluoryl compound loses hydrogen with conversion to the unsaturated bifluorylidene (5). This fact was used to prepare 2,2'-diacetyl-amino-9,9'-bifluorylidene (III). The bifluoryl derivative, II, was dissolved in refluxing biphenyl, whereby

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it was dehydrogenated. After cooling, the biphenyl was removed by extraction with petroleum ether, leaving a mixture of bifluorylidene and unchanged bifluoryl. Attempts to dehydrogenate the bifluoryl by mild oxidizing agents



such as ferric chloride or hydrogen peroxide were not successful. However, it was found that III could be obtained directly by reduction of IV with iron and glacial acetic acid. The double bond was not reduced under these conditions.

The difference in the melting points of the 2,2'-diacetyl-amino-9,9'-bifluorylidene obtained by the two methods may have been due to *cis* and *trans* isomers.

The diacetylaminobifluorylidenes were all converted to the bifluoryl by action of zinc and glacial acetic acid.

#### EXPERIMENTAL

*9-Bromo-2-nitrofluorene*. This compound was prepared according to the method of Korczynski, *et al.* (6).

*2,2'-Dinitro-9,9'-bifluorylidene (IV)*. Fifty-six grams of 9-bromo-2-nitrofluorene was dissolved in 400 cc. of acetone and the solution cooled to room temperature. Eleven grams of potassium hydroxide in 100 cc. of methanol and 50 cc. of acetone were added slowly with stirring. The orange-red precipitate which began forming immediately was allowed to settle, filtered off, washed and dried. It weighed 44 g. The melting point was over 360° as reported by Korczynski (6).

*2,2'-Dihydroxylamino-9,9'-bifluoryl (V)*. Thirty grams of 2,2'-dinitro-9,9'-bifluorylidene was suspended in 500 cc. of ethanol and 200 cc. of dioxane containing 10 g. of calcium chloride in 75 cc. of water. Eighty grams of zinc dust was added, and the whole thoroughly mixed and refluxed for 2 hours. The excess zinc was filtered off and the filtrate diluted with 2 l. of water. The tan-yellow precipitate weighed 22 g. It reduced Fehling's solution and reddened on the copper block but did not melt below 360°. It could be recrystallized from benzene or xylene.

*Anal.* Calc'd for  $C_{26}H_{20}N_2O_2$ : N, 7.14; Mol. wt. 392.

Found: N, 7.31; Mol. wt. 383.

*2,2'-Diacetyl-amino-9,9'-bifluoryl (II)*. *A. By reduction of IV*. One gram of 2,2'-dinitro-9,9'-bifluorylidene was refluxed with 4 g. of zinc dust in 50 cc. of glacial acetic acid for 2½ hours. Then 2 cc. of acetic anhydride was added and the mixture refluxed for 15 minutes. After filtration and dilution of the filtrate with water a white precipitate formed. This after drying weighed 0.9 g. and melted at 255° (block). After recrystallization from dioxane or acetic acid it melted at 328° (block).

*Anal.* Calc'd for  $C_{30}H_{24}N_2O_2$ : N, 6.30. Found: N, 6.06.

*B. By reduction of V*. Thirty grams of the hydroxylamino compound was dissolved in 400 cc. of glacial acetic acid and 40 g. of zinc dust added in portions over a period of one hour. Then 40 cc. of acetic anhydride was added dropwise. After filtration and dilution of the filtrate to 2 l. with water, 34 g. of crude 2,2'-diacetyl-amino-9,9'-bifluoryl was obtained, m.p. 255°.

*2,2'-Diacetyl-amino-9,9'-bifluorylidene (III)*. *A. By fusion of II*. A mixture of 14 g. of 2,2'-diacetyl-amino-9,9'-bifluoryl and 60 g. of biphenyl was heated in a metal bath for 15 minutes at a temperature sufficient to keep the biphenyl refluxing. The red mixture which formed was extracted in a Soxhlet with petroleum ether for two hours to remove the biphenyl. The red residue weighed 14 g. Of this amount 2 g. was soluble in benzene and the fine carmine-red crystals melted at 190° after recrystallization from benzene.

*Anal.* Calc'd for  $C_{30}H_{22}N_2O_2$ : N, 6.33. Found: N, 6.27.

The material insoluble in benzene yielded the unchanged bifluoryl after two recrystallizations from glacial acetic acid using charcoal. A trace of the higher-melting isomer (see B below) was also obtained.

*B. By reduction of IV*. One gram of IV was refluxed with 4 g. of iron powder and 40 cc. of glacial acetic acid for 1½ hours. After adding 1 cc. of concentrated hydrochloric acid and boiling for one minute the mixture was filtered. The solution was refluxed with 5 cc. of acetic anhydride for 15 minutes, diluted to 250 cc. with water, and acidified with 10 cc. of concentrated hydrochloric acid to dissolve basic iron salts. The precipitate weighed 1.2 g., and melted at 240°. After recrystallizing from glacial acetic acid the dark red microcrystals melted at 264-265°.

*Anal.* Calc'd for  $C_{30}H_{22}N_2O_2$ : N, 6.33; Mol. wt. 442. Found: N, 6.05; Mol. wt. 433.

It appeared probable that some of the lower-melting isomer (see A above) was present in the crude material and mother liquors but it was not obtained in a pure state.

#### SUMMARY

In order to obtain experimental evidence of Pinck's hypothesis of the genesis of cancer, the synthesis of the possible carcinogens, 2,2'-diacetylamino-9,9'-bifluoryl and 2,2'-diacetylamino-9,9'-bifluorylidene, has been accomplished.

The carcinogenetic properties of these compounds are being investigated by Dr. H. P. Morris of the National Cancer Institute and will be reported by him subsequently.

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