Journal of Fluorine Chemistry, 8 (1976) 513 – 525

Received: July 9, 1976

ACTIVE SITES IN DIBENZOPYRENES: SYNTHESIS AND STUDIES OF 3-FLUORO- AND 2,10-DIFLUOROBENZO(RST)PENTAPHENE

ELIAHU BOGER, ROBERT F. O'MALLEY and D. J. SARDELLA

Department of Chemistry, Boston College, Chestnut Hill, Mass. 02167 U.S.A.

SUMMARY

9-Fluoro-7H-benz(de)anthracene-9-one (9-fluorobenzanthrone) was prepared from 2-fluoro-9-anthrone by the Bally-Scholl reaction. The identity and structure of the compound were demonstrated by M.S. and C-13 NMR analysis. Its condensation with phenylacetonitrile, followed by a sequence of reactions leading to the hexacyclic aromatic system, yielded 3-fluorobenzo(rst)pentaphene; with p-fluorophenylacetonitrile, 2,10-difluorobenzo(rst)pentaphene was obtained. The results of testing of the two compounds for carcinogenic activity support the recent hypotheses that metabolic activation and binding of the parent hydrocarbon occur on the lateral rings rather than the K regions.

INTRODUCTION

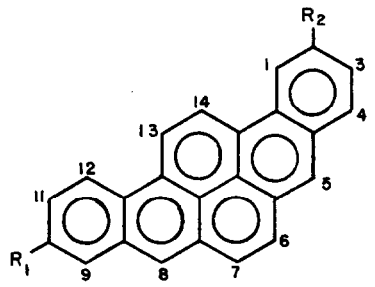
Fluorine-Substitution in benzene rings of various aromatic carcinogens have proved to be a useful tool in investigating their active sites and sites of metabolic transformation. The advantage of the fluorine atoms is its small size, its strong bond with carbon and its resistance to metabolism *in situ*, as contrasted with other groups [1].

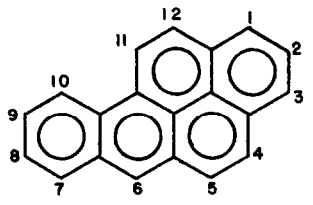
The rationale behind the use of fluoro derivatives of carcinogens in the study of their active sites, introduced by Miller and Miller [2,3] and by Newman [1] is that if a fluorinated molecule is biologically active, the position occupied by the F atom is not directly involved in the biological activity under test. On the other hand, if a fluorinated derivative is not active, then the position blocked by F may be assumed to participate in the activity of the non-fluorinated parent molecule. This rationale has been successfully applied in the study of the carcinogenic 2-acetylaminofluorene [4,5], 4-dimethylaminoazobenzene [6], 7-methylbenz(a)anthracene [2], 7,12-dimethylbenz(a)anthracene [7] and 6,8-dimethylbenz(a)anthracene [8]. In these compounds almost all the various monofluoro isomers have been prepared and tested for carcinogenic activity. So far, very little has been done along these lines on larger polycyclic carcinogens, such as dibenzanthracenes [9] and benzo(a)pyrene [10].

In this paper we report the synthesis of two fluoro derivatives of benzo(rst)pentaphene (also known as dibenzo(a,i)pyrene or 3,4,9,10-dibenzopyrene, DP,I) and the study of their biological activities. DP is a potent carcinogen and has been used in the production of tumors in mice for experimental chemotherapeutic studies [11]. It occurs in tobacco and marijuana smoke [12,13] as well as in exhaust fumes etc., and was recently found to cause lung cancer in hamsters [14]. In a review article on dibenzopyrenes by Buu-Hoi, which appeared in 1964 [15], the importance of the mesophenanthrenic "K-regions" of these compounds in the process of carcinogenic activity was stressed. Indeed, of the five dibenzopyrene isomers, only the one devoid of a "K-region" (namely, 1,2,6,7-dibenzopyrene) is noncarcinogenic. However, in 1967 studies of the ozonolysis of DP failed to find any correlation between the Pullman's theoretical K-

region values [16] and the reactivity of these sites to ozone, known to add preferentially to reactive bonds [17]. More recently, Waterfall and Sims have reported that I does not react with the "K-region" reagent OsO_4, but is metabolized to dihydrodiols and phenols (of unknown structure) by microsomal enzymes [12]. It also requires enzymatic activation in order to bind to DNA and proteins. These authors therefore suggest that metabolic activation of I takes place at the 1,2-, 3,4-, 9,10- and 11,12-bonds rather than at the 6,7- and 13,14- "K-regions" [ref. 12, p. 2481]. This assumption has found support in more recent reports on the involvement of the corresponding 7,8- and 9,10-bonds in benzo(a)pyrene metabolism [18,19] and on the high mutagenicity of 7,8-dihydroxy-9,10-epoxy 7,8,9,10-tetrahydrobenzo(a)pyrene metabolite which is believed to be the main ultimate form leading to carcinogenesis [20,21].

We have approached this problem by synthesizing fluoro derivatives of I in which the fluorine atoms block sites on lateral rings, and testing them for carcinogenic activity. Our findings that the 3-fluoro derivative II is a very weak carcinogen and the 2,10-difluoro III is noncarcinogenic provide strong support to the Waterfall-Sims assumption concerning the site of activation of DP and therefore also to the recent hypothesis on the structure of the ultimate carcinogenic metabolite of benzo(a)pyrene [20,21].

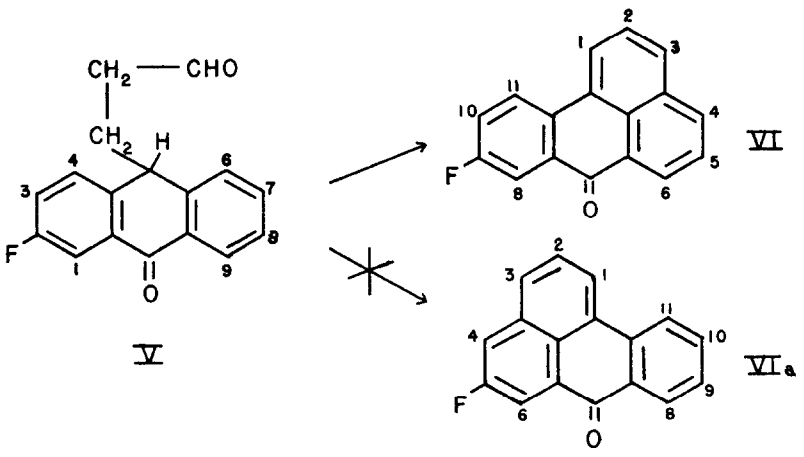




I $R_1 = R_2 = H$ II $R_1 = F$, $R_2 = H$ III $R_1 = R_2 = F$ IV

RESULTS

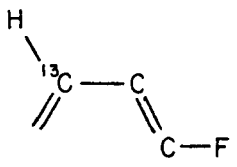
The key intermediate for the synthesis of both II and III is 9-fluoro-7H-benz(de)anthracene-7-one (9-fluorobenzanthrone, VI). This compound was prepared from the known 2-fluoro-9-anthrone [22] by condensation with glycerol in H_2SO_4 (Bally-Scholl reaction). The mechanism of this reaction is believed to be an addition of the 10-carbon of the anthrone to the double bond of the acrolein to form the adduct V which then cyclizes to the benzanthrone [23]. Since the position meta to the F atom is highly deactivated [24] we assumed that the product obtained was 9-fluorobenzanthrone (VI) rather than the alternative isomer, 5-fluorobenzanthrone VIa.

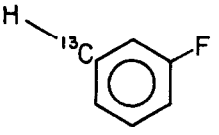


This was confirmed by ^{13}C nmr analysis. (Spectra were taken on a Varian Associates Model CFT-20 nmr spectrometer.)

The coupled ^{13}C nmr spectrum (taken at 20 MHz in $CDCl_3$) is quite complex, due to the presence of both ^{13}C-H and

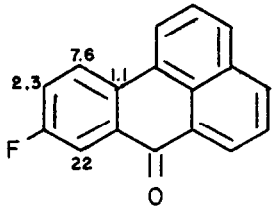
^{13}C-^{19}F couplings. Proton noise decoupling removes all the ^{13}C-H couplings, leaving only the ^{13}C-^{19}F couplings, which appear as four doublets, at 111.4 (J_{CF}=22.0 Hz), 118.7 (J_{CF}= 22.9 Hz), 121.9 (J_{CF}=1.1 Hz) and 123.2 (J_{CF}=7.6 Hz) ppm downfield from TMS. The ca. 23 Hz splittings arise from two-bond coupling between ^{13}C and the fluorine ortho to it. The 7.6 Hz splitting arises from coupling between ^{13}C and a fluorine three bonds removed (meta to it). In the coupled spectrum, this latter doublet is further split by a one-bond ^{13}CH coupling (J_{CH}=168 Hz). Thus, it corresponds to a structural feature

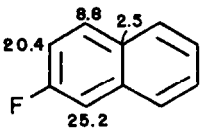


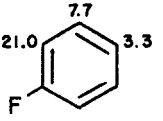


Only VI contains such a feature, and on this basis its structure can be assigned.

The ^{13}C-^{19}F couplings in fluorobenzanthrone and model systems are [25]:

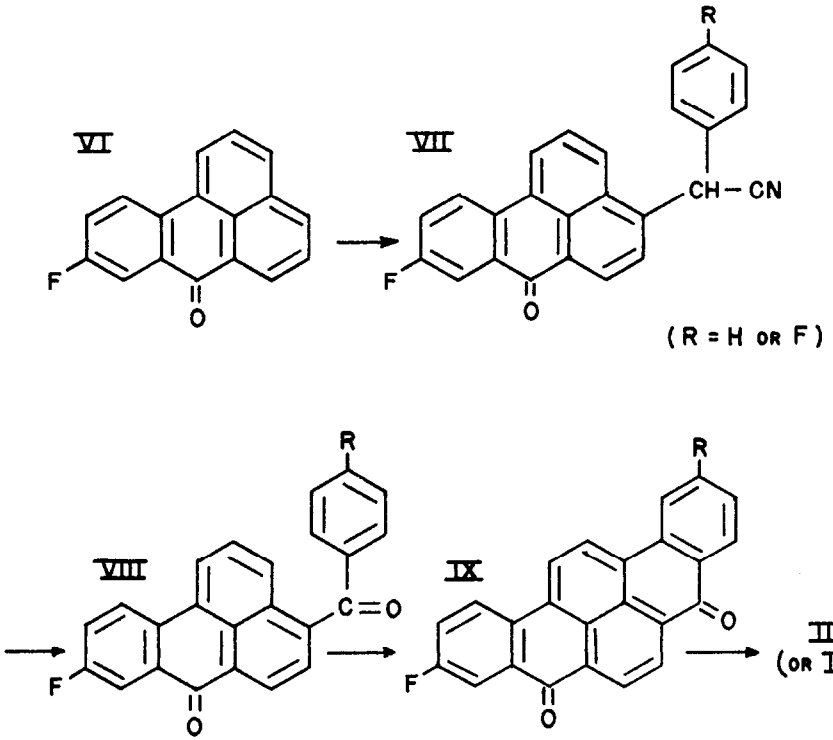






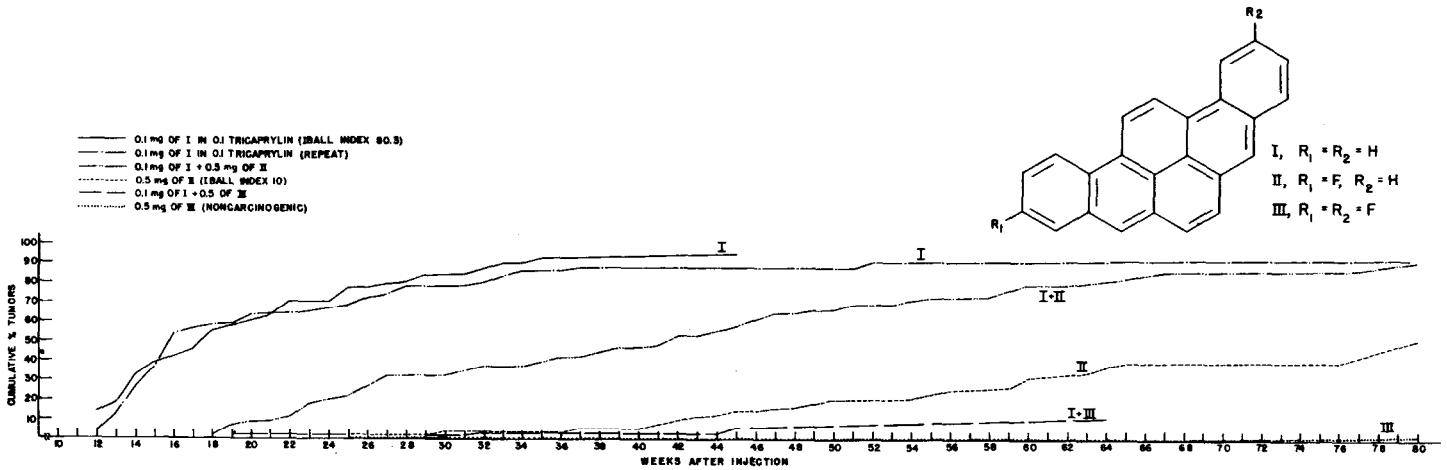
A similar case of meta deactivation by F was the cyclization of β-(9,10-dihydro-3-fluoro-9-anthryl)butyric acid studied by Blum and Bergmann [10].

Condensation of 9-fluorobenzanthrone (VI) with phenylacetonitrile in isopropanolic KOH gave 4-cyanobenzyl-9-fluoro-7H-benz(de)anthracene-7-one (VII, R=H) which was converted to the 4-benzoyl derivative (VIII, R=H) by oxidative hydrolysis. Cyclization of the latter compound followed by reduction of the fluoro dione (IX, R=H) led to compound II. In a similar way, compound III was obtained from VI by condensation with p-fluorophenyl acetonitrile followed by the same sequence of reactions.



The biological assays, which will be published elsewhere [26], are summarized on chart 1. The carcinogenic activity of II expressed by the Iball index [27] is about 10, compared to 80-81 for I. III is completely noncarcinogenic. It should be noted that II and III can antagonize the carcinogenic effect of I.

CHART I. CARCINOGENIC ACTIVITY OF FLUORO DERIVATIVES OF BENZO (RST) PENTAPHENE (3,4,9,10-DIBENZPYRENE, I) AND THEIR ANTAGONISTIC EFFECT ON PARENT HYDROCARBON (I) IN C57BL/6 MALE MICE (BY S.C. INJECTIONS)



EXPERIMENTAL*

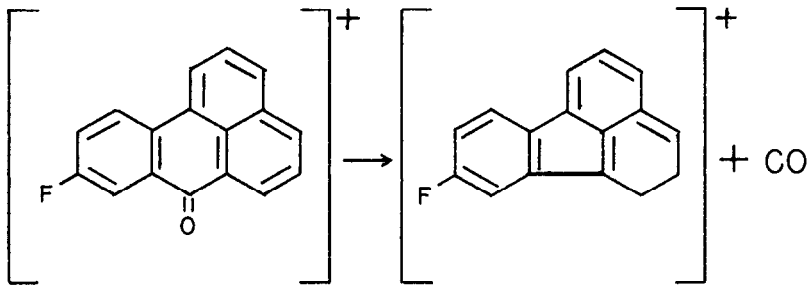
9-Fluoro-7H-benz(de)anthracene-7-one (VI) (n.c.)

The starting material, 2-fluoro-9-anthrone was prepared from the commercially available o-(4-fluorobenzoyl)benzoic acid (Aldrich) according to the procedure of Bergmann et al. [22].

10.6 g (50 mM) of 2-fluoro-9-anthrone, m.p. 145-7°, and 150 g conc. H_2SO_4 were placed in a 1-liter flask equipped with a condenser, a drying tube and a mechanical stirrer. To this mixture were added 20 ml of a 1:1 solution of glycerol and water during 30 min while stirring. During the addition the temperature of the reaction mixture rose to 90°C. After slowly heating the mixture to 120° (for 1.5 hours) the temperature was maintained at this level for another three hours. After cooling to 70°, the mixture was poured into 550 ml of boiling water with constant stirring. The suspension formed was boiled for a few minutes and allowed to stand overnight. After boiling with 170 ml 10% NaOH for 30 minutes, the black solid was filtered, washed with water and dried. It was then extracted with ether in a Soxhlet apparatus for 48 hours. 6.2 g of a yellow solid material, m.p. 158-161° were obtained (50% yield). M.S. analysis revealed the presence of a small amount of 2-fluoroanthraquinone. Purification was achieved by fractional vacuum sublimation followed by recrystallization from isopropanol. M.p. 172°. Anal. Calcd. for $C_{17}H_9FO$: C, 82.30; H, 3.62; F, 7.64%. Found: C, 82.85; H, 3.60; F, 7.42%. In chloroform solution the compound showed

*All melting points are uncorrected. Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. and by Schwarzkopf Laboratory, Woodside, N.Y.

carbonyl absorption at 1660 cm^{-1}. Mass spectrum showed peaks at 248 (fluorobenzanthrone) and 220, by the loss of CO:



Similar observation with benzanthrone was made by Beynon and Williams [28].

<u>α-Phenyl-7-oxo-7H-benz(de)anthracene-4-acetonitrile-9-fluoro</u> (n.c.)
<u>(4-cyanobenzyl-9-fluoro-7H-benz(de)anthracene-7-one, VII, R=H)</u>

24.2 ml Isopropanol and 7.6 g of powdered potassium hydroxide were mixed with good agitation and cooled to room temperature. Into this mixture there were added 2.5 g of 9-fluorobenzanthrone (VI) followed by 10 ml of phenyl acetonitrile (benzylcyanide). The mixture was heated to 45°C and air was bubbled through for 5 hours, keeping the temperature between 40 and 45°. After cooling to room temperature, 7.2 ml of glacial acetic acid were added, and the mixture was stirred at 30-35° for 5 hours. The solid material was filtered by suction, boiled with methanol and filtered. After recrystallization from ethylene glycol monomethyl ether 2.0 g of VII, R=H were obtained. M.p. 224°. Anal. Calcd. for $C_{25}H_{14}FNO$: C, 82.63; H, 3.86; F, 5.24; N, 3.86%. Found: C, 82.69; H, 3.73; F, 5.04; N, 3.62%.

4-Benzoyl-9-fluoro-7H-benz(de)anthracene-7-one (VIII, R=H) (n.c.)

A mixture of 3.1 g sodium acetate, 2.75 g sodium dichromate, 2.0 g VII, R=H and 14 ml glacial acetic acid were heated on a boiling water bath for three hours. After cooling, 7 ml of water were added slowly (to avoid the precipitation of large particles). The precipitate was filtered and washed with hot water until a colorless filtrate was obtained. Recrystallization from benzene petrol ether yielded 1.6 g (80%), m.p. 220°. Anal. Calcd. for $C_{24}H_{13}FO_2$: C, 81.60; H, 3.97; F, 5.38%. Found: C, 82.22; H, 3.69 F, 5.51%.

3-Fluorobenzo(rst)pentaphene-5,8-dione (IX, R=H) (n.c.)

A mixture of 0.9 g KCl, 0.9 g NaCl and 10.2 g technical aluminum chloride (Eastman) was heated to 125°C in a small Erlenmeyer flask. (If pure aluminum chloride is used, no reaction takes place. See ref. 29, p. 110.) When the mixture melted, 1.6 g of VIII, R=H were added, followed by 0.6 g m-nitrobenzoic acid. Heating was continued for four hours, after which the mixture was boiled with a solution of 5 ml conc. HCl and 50 ml water. The solid material was filtered and boiled in a solution of 5 ml HCl, 5 ml ethanol and 25 ml water. 1.5 g of the dione (95% yield) were obtained. Red crystals, m.p. 395° (from xylene). Anal. Calcd. for $C_{24}H_{11}FO_2$: C, 82.27; H, 3.16; F, 5.42%. Found: C, 81.59; H, 3.23; F, 5.41%.

3-Fluorobenzo(rst)pentaphene II (n.c.)

The dione IX, R=H was reduced with Zn dust in a melt of $ZnCl_2$ and NaCl according to the procedure of Unseren and Fieser [11]. Yield, 30%. Yellow crystals, m.p. 265°, were

obtained by chromatography on alumina. Anal. Calcd. for $C_{24}H_{13}FO_2$: C, 89.99; H,4.06; F, 5.95%. Found: C, 89.94; H, 4.04; F, 6.29%. M.S. Showed a peak at 320. U.V. Spectrum was very similar to that of benzo(rst)pentaphene [23, p. 156].

The difluoro derivatives (R=F) were prepared in a similar way:

α-(p-Fluorophenyl)-7-oxo-7H-benz(de)anthracene-4-acetonitrile-9-fluoro- (VII, R=F) (n.C.) was prepared from VI and p-fluorobenzyl cyanide (Aldrich). M.p. 161-3° (from ethylene glycol monomethyl ether). Yield, 50%. Anal. Calcd. for $C_{25}H_{13}F_2NO$: C, 78.73; H 3.43; F, 9.96%; N, 3.69%. Found: C, 78.56; H, 3.72; F, 10.39; N, 3.54%.

4-(p-Fluorobenzoyl)-9-fluoro-7H-benz(de)anthracene-7-one (VIII), R=F) (n.c.)

M.p. 223-4° (from benzene-petrol ether). Yield, 80%. Anal. Calcd. for $C_{24}H_{12}F_2O_2$: C, 77.84; H, 3.24; F, 10.26%. Found: C, 77.55; H, 3.12; F, 10.29%.

2,10-Difluorobenzo(rst)pentaphene-5,8-dione (IX, R=F) (n.c.)

M.p. 390° (from xylene). Yield, 90%. Anal. Calcd. for $C_{24}H_{10}F_2O_2$: C, 78.26; H, 2.74; F. 10.32. Found: C, 78.36; H, 2.96; F, 10.33%.

2,10-Difluorobenzo(rst)pentaphene (III) (n.c.)

Golden-yellow crystals, m.p. 261-2°. Anal. Calcd. for $C_{24}H_{12}F_2$: C, 85.21; H, 3.57; F, 11.22%. Found: C, 84.99; H, 3.81; F, 10.72%. M.S. shows peak at 338. U.V. Spectrum is similar to those of I and II.

ACKNOWLEDGEMENT

The authors are indebted to Prof. George Vogel of Boston College for performing and interpreting the mass spectra. The technical assistance of Mr. William Brenner is gratefully appreciated.

This investigation was supported by grants number CA10069 and IR01CA18959, awarded by the National Cancer Institute, DHEW.

REFERENCES

1 M.S. Newman, D. MacDowell and S. Swaminathan, J. Org. Chem., 24 (1959) 509.
2 E.C. Miller and J.A. Miller, Cancer Res., 20 (1960) 133.
3 J.A. Miller and E.C. Miller, Cancer Res., 23 (1963) 229.
4 E.C. Miller, T.L. Fletcher, A. Margreth and J.A. Miller, Cancer Res., 22 (1962) 1002.
5 J.C. Arcos and M.F. Argus, Chemical Introduction of Cancer, Vol. II B, Academic (New York) 1974, p. 56-58.
6 J.A. Miller, E.C. Miller and G.C. Finger, Cancer Res., 17 (1957) 387; see also ref. 5, p. 146-155.
7 M.S. Newman and R.F. Cunica, J. Med. Chem., 15 (1972) 323. See footnotes on p. 324 (unpublished results by Miller and Miller and by C.B. Huggins' group).
8 M.S. Newman, J. Cecil and W. Hung, J. Med. Chem., 15 (1972) 569.
9 J. Blum and E.D. Bergmann, J. Org. Chem., 32 (1967) 342.
10 J. Blum and E.D. Bergmann, J. Org. Chem., 32 (1967) 344.
11 E. Unseren and L.F. Fieser, J. Am. Chem. Soc., 27 (1962) 1386.
12 J.F. Waterfall and P. Sims, Biochem. Pharmacol., 22 (1973) 2469.

13 M. Novotny, M.L. Lee and K.D. Bartle, Chem. Eng. News, 53 (1975) 27.

14 A. Sellakumar and P. Shubik, J. Nat. Cancer Inst., 53 (1974) 1713.

15 N.P. Buu-Hoi, Cancer Res., 24 (1964) 1511.

16 A. Pullman and B. Pullman, Adv. Cancer Res., 3 (1955) 117 Academic (New York).

17 E.J. Moriconi and L. Salce, J. Org. Chem., 32 (1967) 1263.

18 H.W.S. King, M.H. Thompson and P. Brookes, Cancer Res., 35 (1975) 1263.

19 Thomas Meehan, D. Warshawaky and M. Calvin, Proc. Nat. Acad. Sci. U.S.A., 78 (1976) 1117; T. Meehan, K. Straub and M. Calvin, ibid., 1437.

20 H. Yag, O. Hernandez and D.M. Jerina, J. Am. Chem. Soc., 97 (1975) 6882.

21 P. Sims, P.L. Grover, A. Swaisland, K. Pal and A. Hewer, Nature (London), 252 (1974) 326.

22 E.D. Bergmann, J. Blum and S. Butanaro, J. Org. Chem., 26 (1961) 3211.

23 E. Clar, Polycyclic Hydrocarbons, Vol. II, Academic (New York) 1974 p. 386.

24 A.E. Pavlath and A.L. Leffler, Aromatic Fluorine Compounds, Reinhold (New York) 1962, p. 40.

25 F.J. Weigert and J.D. Roberts, J. Am. Chem. Soc., 93 (1971) 2361.

26 E. Boger, R.F. O'Malley and A. Treger, Submitted for publication.

27 D.B. Clayson, Chemical Carcinogenesis, Brown and Co. (Boston) 1962, p. 96.

28 J.H. Beynon and A.E. Williams, Applied Spectroscopy, 14 (1960) 156.

29 E. Boger and P. Bernfeld, J. Labelled Compd., 1 (1965) 109.