THE CARCINOGENICITY OF TWO DIAZADIBENZOPYRENES

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Summary.—1,12-Diazadibenzo(a,i)pyrene (I), an isostere of the extremely potent carcinogen dibenzo(a,i)pyrene, also displays carcinogenicity although to a considerably lesser degree than the latter compound. While dibenzo(a,h)pyrene is known to be distinctly less active than dibenzo(a,i)pyrene, surprisingly 4,11-diazadibenzo(a,h)pyrene (II) shows a greater activity than I. Another hexacyclic diaza-hydrocarbon, 4,12-diazadibenzo(g,p)chrysene (III), which is devoid of a meso-phenanthrenic region, proved totally inactive.

Dibenzo(a,i)pyrene (IV) is the most potent carcinogenic hydrocarbon known so far (Lacassagne et al., 1957) in respect of the speed of its action and the high incidence of tumours (Lacassagne, Buu-Hoï and Zajdela, 1958; Waravdekar and Ranadive, 1958); the isomeric hydrocarbon dibenzo(a,h)pyrene (V) is also a highly active carcinogen although to a lesser degree (Bachmann et al., 1937; Shabad, 1938; Badger et al., 1940; Lacassagne et al., 1958). It was interesting to examine whether, and in which direction, the oncogenicity of these 2 aromatic hydrocarbons would be modified by replacement of the 2 external benzene rings by equivalent pyridine ones. Two compounds which meet this structural requirement are 1,12-diazadibenzo(a,i)-pyrene (I), which is isosteric with the hydrocarbon IV, and 4,11-diazadibenzo(a,h)pyrene (II), which is isosteric with the hydrocarbon V. In these diaza-hydrocarbons (Dufour, Buu-Hoï and Jacquignon, 1967), the two meso-phenanthrenic regions considered important for the carcinogenicity of these 2 hexacyclic hydrocarbons (Chalvet and Chalvet, 1955; Pullman and Pullman, 1955) are intact; 6,7-diazadibenzo(a,i)pyrene (VI), in the

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molecule of which, aza-substitution involves one of these K-zones, is totally inactive (Homburger, Treger and Boger, 1968).

Compounds I and II were tested for carcinogenic activity in mice; a third diaza-hydrocarbon, 4,12-diazadibenzo-(g,p)chrysene (III), isomeric with I and II but possessing no free meso-phenanthrenic region, was included in this study for the sake of comparison.

**MATERIALS AND METHODS**

The substances tested were synthesized in our laboratory at Gif-sur-Yvette; their purity (100%) and structure were controlled by physicochemical methods (microanalyses, NMR, etc.).

Two strains of mice, Swiss (Carshalton) and Radium Institute XVII nc/ZE (♀ and ♂), aged 3 to 4 months, were used. As in all our carcinogenicity testing experiments, they were given 3 injections, in the subcutaneous tissue of the right flank, of 0.6 mg of the substance under test, dissolved in 0.2 ml sterile, neutral olive oil, one month elapsing between each injection. The controls (120 Swiss mice and 680 XVII nc/ZE mice) received the solvent only. The animals which did not develop tumours were kept for over 600 days after the start of the experiments; those which developed a sarcoma in situ were sacrificed when the diameter of the tumour had reached about 15 mm. For histopathological examination, the tumours were fixed in Bouin's solution and embedded in paraffin, and the sections stained by the trichrome method (haemalum/eosin/saffron). Post-mortem examinations were performed on all the animals and a search made for possible remote tumours.

Compound I was tested in 28 mice and compound II in 27; compound III was tested in 30 mice.

**RESULTS AND DISCUSSION**

The tests for carcinogenicity gave very similar results in the 2 strains of mice. As dibenzo(a,i)pyrene (IV), inoculated

| Table. — Sarcomagenic Activity of Compounds I and II |
|----------------------------------------------------|
| **Strain Swiss (Carshalton)**                      |
| **Strain XVII nc/ZE (Radium Institute)**           |
| **Day killed** | **Latency period of sarcomata** | **Day killed** | **Latency period of sarcomata** | **Day killed** | **Latency period of sarcomata** | **Day killed** | **Latency period of sarcomata** |
| ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
|---|---|---|---|---|---|---|---|
| 146 | 122 | 154 | 142 | . | 131 | 100 | 162 | 138 |
| 230 | 192 | 185 | 142 | . | 190 | 152 | 173 | 150 |
| 251 | 207 | 632 | — | . | 195 | 160 | 632 | — |
| 462 | — | 632 | — | . | 210 | 192 | 632 | — |
| 632 | — | 632 | — | . | 632 | — | 632 | — |
| 632 | — | 632 | — | . | 632 | — | 632 | — |

Mean latency time, 154 days. Iball index,* 25. Yield of tumours, 39%.

| 4,11-Diazadibenzo(a,h)pyrene (Compound II) |
|--------------------------------------------|
| **Day killed** | **Latency period of sarcomata** | **Day killed** | **Latency period of sarcomata** | **Day killed** | **Latency period of sarcomata** | **Day killed** | **Latency period of sarcomata** |
| ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
|---|---|---|---|---|---|---|---|
| 181 | 142 | 154 | 108 | . | 182 | 127 | 162 | 124 |
| 181 | 142 | 181 | 139 | . | 173 | 137 | 162 | 124 |
| 181 | 162 | 181 | 145 | . | 182 | 145 | 205 | 182 |
| 181 | 162 | 181 | 162 | . | 182 | 145 | 205 | 182 |
| 204 | 162 | 204 | 162 | . | 205 | 180 | 232 | 203 |
| 284 | 242 | 210 | 175 | . | 262 | 230 | 632 | — |
| 364 | 350 | 632 | — | . | 262 | 230 | — | — |

Mean latency time, 175 days. Iball index,* 53. Yield of tumours, 93%.

* Iball index (sarcoma index) is obtained by dividing the percentage of tumours of animals alive at the appearance of the first tumour by the mean latency period in days and multiplying by 100 (Iball, 1939).
which all sacrifice, the analogues reverse degree indicates that the introduction of earlier gen products (Lacassagne et al., 1958) with, however, a longer latency time (175 days compared with 111 days), which gives the diaza product II an Iball index of 53 compared with 78 for the original hydrocarbon. These results indicate that the introduction of 2 nitrogen atoms in the skeleton of carcinogenic dibenzopyrenes does not suppress their sarcomagenic potency if the two K-regions remain intact. The superiority of II over I in producing tumors is unexpected considering that the situation is the reverse in the case of their hydrocarbon models V and IV.

These 2 examples emphasize the difficulty of making any prediction of the degree of carcinogenicity of nitrogen analogues of hydrocarbons, and point to the likelihood of encountering large numbers of carcinogens among them.

Compound III was completely inactive, all the animals having survived over 675 days without showing any tumor at sacrifice, except for 2 mice, one a Swiss ♀, which died after 472 days with a hepatoma, probably spontaneous, the other, a XVII neo/ZE, ♀, which died without tumor on the 562nd day.

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