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ZOXAZOLAMINE-HYDROXYLASE INDUCING EFFECT OF POLYCYCLIC AROMATIC HYDROCARBONS; RELATIONSHIPS BETWEEN STRUCTURE AND ACTIVITY, AND DEGREE OF CORRELATION WITH CARCINOGENICITY

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Abstract—In continuation of previous work, the zoxazolamine-hydroxylase inducing properties of aromatic hydrocarbons having from three to seven rings have been investigated *in vivo* in young rats by means of the paralysis test; the validity of this test as a gauge for stimulation of enzyme synthesis is confirmed by the antagonistic influence of 6-mercaptopurine. Relationships between molecular structure and enzyme-inducing activity, and the degree of correlation with carcinogenicity, are discussed.

IN THE course of their studies on the chemical stimulation of the synthesis of liver microsomal enzymes, Conney, the Millers, and their coworkers[1] found that certain polycyclic aromatic hydrocarbons are inducers of one such enzyme, zoxazolamine hydroxylase, which converts the paralysing drug zoxazolamine (2-amino-5-chlorobenzoxazole) into an inactive ring-hydroxylated compound. Arcos, Conney, and Buu-Hoï extended this research to several other hydrocarbons,[2] and the present authors found this inducing activity to be particularly frequent among hydrocarbons of specific structures such as naphthacene[3] or chrysene[4] derivatives. It seemed therefore important to conduct a broad study on the relationships between chemical structure and the zoxazolamine-hydroxylase inducing effect in a large number of hydrocarbons of diverse molecular configuration. The inclusion in this study of a sufficient number of both carcinogens and non-carcinogens would also permit a definitive assessment of the degree of correlation between this particular type of biological activity and cancer-inducing properties among hydrocarbons, which might be of help for the understanding of the carcinogenesis process (a similar assessment has already been made in regard to carcinogenic nitrogenous heterocycles[5]).

A convenient pharmacological method for the *in vivo* estimation of increases in zoxazolamine hydroxylase levels is the determination of the duration of zoxazolamine-produced paralysis in young rats pretreated with the potential enzyme inducer, as compared with controls which receive only zoxazolamine, in the same dosage. As shown by Conney *et al.*,[2, 6] the reduction of the duration of paralysis parallels the increase in the zoxazolamine hydroxylase levels found in the liver homogenates. A further advantage of the paralysis test is the possibility it provides for appreciating the overall quantity of inducible zoxazolamine hydroxylase present in the body (it is known that detoxifying oxidative enzymes closely related to, if not identical with, zoxazolamine hydroxylase, such as benzo[*a*]pyrene hydroxylase, exist in a wide variety of tissues other than the liver[7]).

The connection between reduction in the duration of paralysis and enzyme synthesis is demonstrated by the inhibition of this effect by 6-mercaptopurine, which is known to interfere with the biogenesis of protein synthesis-controlling nucleic acids by inhibiting several reactions leading to purine nucleotides.[8]

As well as the 56 polycyclic aromatic hydrocarbons investigated, some of their furan and thiophen isosters, and several of their functional derivatives, were also studied. Some steroids, anthrasteroids, and triterpenes have been included in the present investigation in view of their conceivable conversion *in vivo* into polycyclic hydrocarbons.

TABLE 1. TRI- AND TETRA-CYCLIC HYDROCARBONS AND DERIVATIVES

Substance		Duration of paralysis (min)* Treated	Controls
TRICYCLIC			
2-Phenylanthracene		103 ± 25 (5)	229 ± 22 (14)
9,10-Dimethylanthracene†		329 ± 37 (5)‡	282 ± 58 (10)
1-Aminoanthracene		177 ± 26 (5)‡	220 ± 93 (10)
TETRACYCLIC			
5-Methyl-2,3-dihydro-1H-cyclopent[*b*]anthracene		132 ± 33 (3)	259 ± 51 (12)
2,3-Dihydro-1H-Cyclopent[*a*]anthracene		292 ± 48 (2)‡	242 ± 40 (8)
7*H*-Benzo[*c*]fluorene		77 ± 3 (2)	218 ± 22 (12)
Fluoranthene		285 ± 80 (5)	246 ± 40 (10)
Benz[*a*]anthracene†		33 ± 8 (5) 45 ± 15 (5)	246 ± 40 (10) 238 ± 49 (10)
1,2,3,4,5,6-Hexadeuterobenz[*a*]anthracene†		37 ± 15 (3) 46 ± 13 (5)	238 ± 49 (10) 243 ± 76 (10)
3-Methylbenz[*a*]anthracene		46 ± 26 (5)	243 ± 76 (10)
4-Methylbenz[*a*]anthracene†		28 ± 3 (5)	243 ± 76 (10)
5-Methylbenz[*a*]anthracene†		34 ± 16 (5)	243 ± 76 (10)
6-Methylbenz[*a*]anthracene†		29 ± 11 (5)	243 ± 76 (10)
7-Methylbenz[*a*]anthracene†		20 ± 2 (5)	229 ± 22 (14)
12-Methylbenz[*a*]anthracene†		46 ± 9 (8)	229 ± 22 (14)
7-Phenylbenz[*a*]anthracene		208 ± 88 (6)‡	218 ± 22 (12)
7-Bromobenz[*a*]anthracene†		35 ± 9 (6)	243 ± 76 (10)
7-Formylbenz[*a*]anthracene†		107 ± 12 (5)	218 ± 22 (12)
7,12-Dimethylbenz[*a*]anthracene†	20 mg/kg	94 ± 10 (8)	266 ± 46 (10)
	10 mg/kg	148 ± 26 (8)	266 ± 46 (10)
	5 mg/kg	206 ± 27 (6)	277 ± 57 (10)
	2·5 mg/kg	245 ± 40 (5)‡	277 ± 57 (10)
	1·25 mg/kg	263 ± 50 (5)‡	277 ± 57 (10)
Perdeutero-7,12-dimethylbenz[*a*]anthracene†	20 mg/kg	91 ± 6 (7)	266 ± 46 (10)
	10 mg/kg	124 ± 27 (7)	266 ± 46 (10)
	5 mg/kg	170 ± 45 (6)	277 ± 57 (10)
	2·5 mg/kg	224 ± 30 (5)‡	277 ± 57 (10)
	1·25 mg/kg	243 ± 45 (5)‡	277 ± 57 (10)
7,12-Diphenylbenz[*a*]anthracene		240 ± 37 (6)‡	218 ± 22 (12)
Benzo[*c*]phenanthrene†		336 ± 100 (4)‡	302 ± 61 (10)
Chrysene†		66 ± 12 (5)	282 ± 83 (10)
Benzo[*b*]naphtho[2,3-*d*]furan		46 ± 9 (5)	218 ± 22 (12)
9-Methylthieno[3,2-*b*]phenanthrene		44 ± 10 (5)	234 ± 30 (10)
Benzo[*b*]naphtho[2,1-*d*]thiophene		109 ± 26 (5)	261 ± 52 (8)
Thianaphtheno[3,2-*b*]quinoline		234 ± 87 (5)‡	242 ± 40 (8)
2-Aminobenzo[*b*]carbazole		105 ± 48 (5)	367 ± 97 (8)
2,6-Diethylbenzo[*b*]carbazole		128 ± 48 (5)§	238 ± 60 (5)

* Second figures in each column represent S.D.'s, and figures in parentheses denote number of rats.
† Denotes *in situ* carcinogenicity in this and the other Tables.
‡ $P > 0{\cdot}05$.
§ $0{\cdot}01 < P < 0{\cdot}05$.
For all results except those otherwise marked, $P < 0{\cdot}01$.
Nomenclature of compounds is that of *The Ring Index*, Patterson, Capell and Walker, 2nd Edit., American Chemical Society, Washington, D.C., 1960.

MATERIAL AND METHODS

The animals selected for our experiments were Wistar rats, because their moderate sensitivity towards zoxazolamine makes the determination of paralysis times easier than with the considerably more sensitive Sprague–Dawley strain. 3-month-old male

TABLE 2. COMPOUNDS WITH FIVE AND MORE RINGS

Substance		Duration of paralysis (min)* Treated	Controls
PENTACYCLIC			
Dibenzo[*a,g*]fluorene†	5 mg/kg	30 ± 5 (5)	325 ± 96 (10)
	10 mg/kg	24 ± 4 (5)	325 ± 96 (10)
	20 mg/kg	18 ± 5 (5)	234 ± 30 (10)
	20 mg/kg	22 ± 3 (5)	325 ± 96 (10)
3-Methylcholanthrene†		25 ± 7 (8)	282 ± 46 (10)
Benzo[*b*]fluoranthene†		24 ± 7 (8)	259 ± 51 (12)
Benzo[*k*]fluoranthene†		41 ± 14 (6)	234 ± 30 (12)
8-Methylbenzo[*k*]fluoranthene		28 ± 7 (4)	218 ± 22 (12)
8,9,10,11-Tetrahydrobenzo[*k*]fluoranthene		58 ± 12 (6)	259 ± 51 (12)
8-Oxo-8,9,10,11-tetrahydrobenzo[*k*]fluoranthene		106 ± 14 (6)	319 ± 41 (14)
Perylene		248 ± 42 (5)‡	246 ± 40 (10)
3-Methylperylene		272 ± 28 (2)‡	259 ± 51 (12)
Dibenz[*a,c*]anthracene		14 ± 1 (2)	229 ± 22 (14)
10-Methyldibenz[*a,c*]anthracene†		29 ± 7 (5)	229 ± 22 (14)
		42 ± 14 (7)	319 ± 41 (14)
Picene		85 ± 15 (2)	242 ± 40 (8)
Benzo[*a*]pyrene†		37 ± 7 (5)	246 ± 40 (10)
2-Methylbenzo[*a*]pyrene†		102 ± 25 (6)	190 ± 30 (9)
		114 ± 29 (6)	283 ± 31 (8)
6-Formylbenzo[*a*]pyrene†		180 ± 60 (6)	334 ± 66 (11)
6-Formylbenzo[*a*]pyrene semicarbazone		140 ± 20 (6)	334 ± 66 (11)
4,5-Dimethylbenzo[*a*]pyrene†		109 ± 29 (6)	190 ± 30 (9)
5-Oxo-5H-benzo[*e*]isochromeno[4,3-*b*]indole†		47 ± 7 (6)	261 ± 52 (8)
HEXACYCLIC			
Dibenzo[*b,k*]fluoranthene		36 ± 10 (6)	319 ± 41 (14)
1,2,3,4,9,10,11,12-Octahydrodibenzo[*b,k*]fluoranthene		56 ± 13 (4)	319 ± 41 (14)
7-Methyldibenzo[*a,e*]fluoranthene		183 ± 82 (5)§	323±100 (10)
6,7-Dimethyldibenzo[*a,e*]fluoranthene		255 ± 60 (5)	323±100 (10)
5,6-Dimethyldibenzo[*a,e*]fluoranthene		319 ± 79 (5)	323±100 (10)
14-Phenyldibenzo[*a,e*]fluoranthene		267 ± 50 (5)	323±100 (10)
Indeno[1,2,3-*fg*]naphthacene		175 ± 7 (3)§	256 ± 80 (10)
Benzo[*a*]naphtho[2,1-*g*]fluorene		209 ± 57 (5)‡	234 ± 30 (10)
Benzo[*a*]naphtho[2,3-*g*]fluorene		115 ± 30 (5)	234 ± 30 (10)
Dibenzo[*a,c*]naphthacene		109 ± 37 (5)	320 ± 80 (10)
Benzo[*ghi*]perylene†		304 ± 98 (5)‡	259 ± 51 (12)
Dibenzo[*a,e*]pyrene†		130 ± 30 (5)	242 ± 40 (8)
5-Methyldibenzo[*a,e*]pyrene†		292 ± 26 (5)‡	282 ± 58 (10)
6-Methyldibenzo[*a,e*]pyrene†		233 ± 62 (5)‡	282 ± 58 (10)
Dibenzo[*e,l*]pyrene		194 ± 67 (4)‡	218 ± 22 (12)
Dibenzo[*a,h*]pyrene†		64 ± 6 (2)	319 ± 41 (14)
Dibenzo[*a,i*]pyrene†		65 ± 5 (4)	319 ± 41 (14)
5-Methyldibenzo[*a,i*]pyrene†		112 ± 34 (5)	319 ± 41 (14)
5-Aminodibenzo[*a,i*]pyrene†		34 ± 6 (5)	240 ± 73 (10)
OCTACYCLIC			
Isoviolanthrene		310 ± 49 (5)‡	282 ± 58 (10)
NON-CONDENSED POLYCYCLIC			
1,3,5-Tri(4-biphenylyl)benzene†		254 ± 48 (5)‡	246 ± 40 (10)
1-Bromo-1,2-diphenyl-1-(4-ethylphenyl)ethylene (trans)		237 ± 84 (6)‡	274 ± 81 (12)

* Second figures in each column represent S.D.'s, and figures in parentheses denote number of rats

† Denotes *in situ* carcinogenicity in this and the other Tables.

‡ $P > 0{\cdot}05$.

§ $0{\cdot}01 < P < 0{\cdot}05$.

For all results except those otherwise marked, $P < 0{\cdot}01$.

Nomenclature of compounds is that of ***The Ring Index***, Patterson, Capell and Walker, 2nd Edit., American Chemical Society, Washington, D.C., 1960.

rats weighing between 90 and 130 g were used, and were fed the usual synthetic vitamin-rich diet[9] starting 7 days before the experiments. The compounds under test were administered intraperitoneally (in solution or suspension in neutral corn oil) at the uniform dose of 20 mg/kg unless otherwise stated. Simultaneously, control tests were conducted with rats receiving the solvent only. Both treated animals and controls were given a uniform dose of zoxazolamine (90 mg/kg in corn oil) 24 hr afterwards. Duration of the paralysis was determined and the results assessed statistically by means of variance analysis.

In most cases, the hydrocarbons investigated had already been tested for carcinogenicity in mice;[10] only carcinogenicity *in situ* is considered in the present context,

TABLE 3. STEROIDS, ANTHRASTEROIDS, AND TERPENES

Substance	Duration of paralysis (min)* Treated	Controls
Oestriol	196 ± 44 (5)§	274 ± 81 (12)
d,l-5,7,9,14-Anthrasta-tetraen-17β-ol	266 ± 18 (5)‡	274 ± 81 (12)
Asiaticoside (aqueous suspension)	210 ± 74 (6)§	330 ± 83 (10)
Asiatic acid (aqueous suspension)	233 ± 12 (5)§	343 ± 74 (13)
Madecassic acid (40 mg/kg; aqueous suspension)	200 ± 30 (3)§	302 ± 61 (10)

* Second figures in each column represent S.D.'s, and figures in parentheses denote number of rats.

† Denotes *in situ* carcinogenicity in this and the other Tables.

‡ $P > 0{\cdot}05$.

§ $0{\cdot}01 < P < 0{\cdot}05$.

For all results except those otherwise marked, $P < 0{\cdot}01$.

Nomenclature of compounds is that of *The Ring Index*, Patterson, Capell and Walker, 2nd Edit., American Chemical Society, Washington, D.C., 1960.

TABLE 4. INHIBITION OF ZOXAZOLAMINE-HYDROXYLASE INDUCTION BY 6-MERCAPTOPURINE

	Duration of paralysis (min) Treated	Controls
(*A*) 3-Methylcholanthrene		
3-Methylcholanthrene alone (20 mg/kg)	25 ± 7 (6)	282 ± 46 (10)
3-Methylcholanthrene + 6-Mercaptopurine	50 ± 13 (6)	282 ± 46 (10)
(*B*) 7,12-Dimethylbenz[*a*]anthracene		
7,12-Dimethylbenz[*a*]anthracene alone (20 mg/kg)	91 ± 6 (7)	266 ± 46 (10)
—ibid— + 6-Mercaptopurine	129 ± 45 (5)	266 ± 46 (10)
(*C*) 5-Methylbenzo[*b*]naphtho[2,1-*d*]thiophene		
5-Methylbenzo[*b*]naphtho[2,1-*d*]thiophene alone (20 mg/kg)	31 ± 8 (5)	215 ± 19 (10)
—*ibid*— + 6-Mercaptopurine	127 ± 43 (5)	215 ± 19 (10)

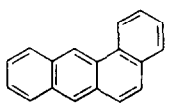
although some of the substances inactive in this manner can produce tumours at remote sites, *via* mechanisms that are probably indirect (e.g. oestriol, which can induce mammary adenocarcinomas).

Experiments to demonstrate the antagonistic effect of 6-mercaptopurine on enzyme induction were performed as follows: the rats received a daily oral dose of 50 mg/kg during 5 days prior to the injection of the enzyme-inducing substance; zoxazolamine was given in the usual way 24 hr later. In the dose-level and under the conditions used

here, 6-mercaptopurine did not on its own show any effect on the duration of the zoxazolamine-induced paralysis; this minimises the likelihood that the inhibiting action of 6-mercaptopurine on the enzyme induction results from a significant lowering of the cofactor NADP levels in the body.[11]

DISCUSSION OF RESULTS

The main feature to emerge from the results obtained in the present investigation is that although zoxazolamine-hydroxylase inducing activity is widely encountered among condensed aromatic hydrocarbons having four to six rings, this property is at the same time highly specific in respect of molecular structure. For instance, in the group of compounds possessing *four* benzene rings, benz[*a*]anthracene (I) and naphthacene[2, 3] (II) are highly active and chrysene (III) fairly so, whereas their isomer, benzo[*c*]phenanthrene (IV) is totally ineffective. Similarly, in the group of hydrocarbons with *five* benzene rings, perylene (V) has no enzyme-inducing properties,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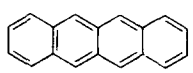
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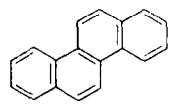
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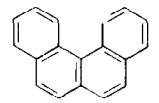
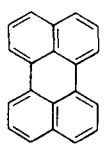

(IV)

whilst dibenz[*a,c*]anthracene (VI), dibenz[*a,h*]anthracene[2] (VII), dibenz[*a,j*]anthracene (VIII), and benzo[*a*]pyrene (IX) are all extremely active, with picene (X), a mildly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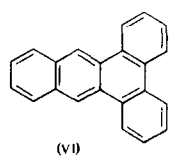
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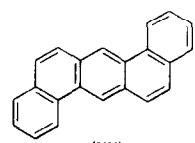
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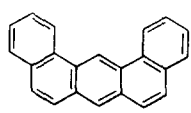
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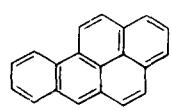
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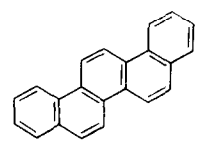
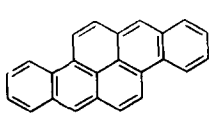

(X)

active compound, occupying an intermediary position. Among the hydrocarbons with *six* benzene rings, also highly demonstrative of the effect of molecular shape on enzyme-inducing properties is the potency of dibenzo[*a,h*]pyrene (XI) and dibenzo-[*a,i*]pyrene (XII; R = H) (both quite active in spite of their poor solubilities), contrasting with the lack of activity found in the isomeric dibenzo[*e,l*]pyrene (XIII) and in benzo[*ghi*]perylene (XV), with dibenzo[*a,e*]pyrene (XIV) and dibenzo[*a,c*]naphthacene (XVI) making the transition between the potent and the inactive compounds.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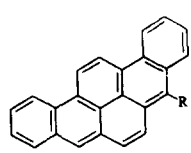

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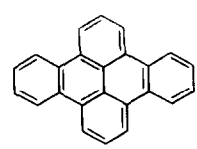
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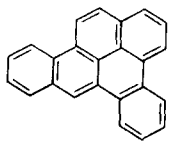

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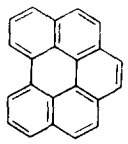

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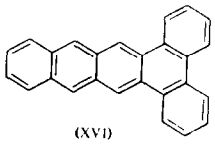
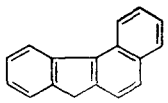


(XVI)

The data concerning hydrocarbons possessing one pentagonal ring are equally informative; activity is present (often to a considerable extent) in compounds whose molecules derive from those of potent benzenoid hydrocarbons by replacement of a benzene ring by a pentagonal one. This is the case of benzo[*c*]fluorene (XVII), and especially of dibenzo[*a*,*g*]fluorene (XVIII) which ranks among the most active enzyme



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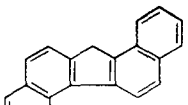
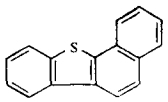
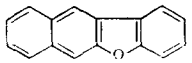
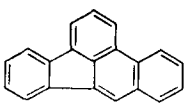


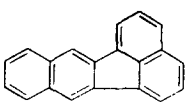


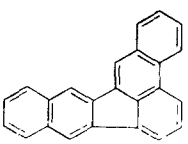


inducers discovered so far (as seen from Table 1, it is at least four times as potent as benzo[*a*]pyrene, taken as a standard); similarly, benzo[*b*]fluoranthene (XXI), benzo[*k*]-fluoranthene (XXII), and dibenzo[*b*,*k*]fluoranthene (XXIII) are all excellent inducers. Activity is also conserved when the pentagonal ring concerned is a thiophene [for



(XXI)



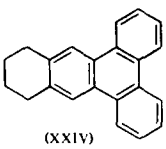


instance in naphtho[1,2-*b*]thianaphthen (XIX)] or a furan ring [as in benzo[*b*]naph-tho[2,3-*d*]furan (XX)].

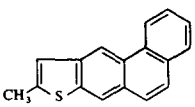
Effects of substituents. As with the polycyclic heterocyclic molecules investigated earlier,[5] introduction of electron-withdrawing substituents diminishes the activity (e.g. in the case of 7-formylbenz[*a*]anthracene and 6-formylbenzo[*a*]pyrene), whereas that of the strong electron-donating amino group enhances it, as is the case with 5-aminodibenzo[*a*,*i*]pyrene (XII; R = NH_2). The influence of methyl substitution depends on the nature of the basic hydrocarbon and on the number of the methyl groups. In the benz[*a*]anthracene series, for instance, all the six monomethylated derivatives are practically as active as the parent hydrocarbon, but the 7,12-dimethyl-derivative is definitely less so; in the more condensed types of hydrocarbons such as benzo[*a*]pyrene and the dibenzopyrenes (XII; R = H) and (XIV), introduction of even one methyl group is already detrimental to the activity. Substitution with the bulky phenyl group is also strongly deactivating, even in the simple benz[*a*]anthracene series.

A particularly interesting kind of substitution examined here is the replacement of hydrogen by deuterium: it can be seen from the data in Table 1 concerning a hexa-deuterobenz[*a*]anthracene and perdeuterated 7,12-dimethylbenz[*a*]anthracene, that these compounds are as active as their non-deuterated analogues.

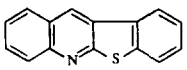
The strong dependence of enzyme-inducing activity on the presence of an adequate electronic pattern in the molecule is emphasised by the effect brought about by replacing a strategically essential aromatic ring by a hydrogenated one. Thus, in passing from dibenz[*a*,*c*]anthracene (VI), an extremely powerful inducer, to 10,11,12-13-tetrahydrobenz[*a*,*c*]anthracene (XXIV) which no longer possesses a *meso*-anthra-



(XXIV)



(XXV)



(XXVI)

cene region (L-zone, in Pullman's terminology[12]), there occurs a total loss of biological activity. This suggests that the biochemical action of dibenz[*a*,*c*]anthracene and similar anthracene hydrocarbons which leads to *enzyme induction is likely to involve the L-zone*. This is supported by the strong activity of all the tetra- and penta-cyclic hydrocarbons which possess such a region, and by the previous[5, 13, 14] and present observations on heterocycles with analogous molecular characteristics; it is remarkable, for instance, that the methyl derivative (XXV) of thieno[3,2-*b*]phenanthrene, which has an L-zone very similar to the one in benz[*a*]anthracene, is practically as active as the methyl homologues of this last. In the case of active hydrocarbons such as chrysene, picene, benzo[*b*]fluoranthene, or dibenzo[*a*,*g*]fluorene, the role of the L-zone is probably assumed by the *meso*-phenanthrene region (or K-zone[12]), or another molecular area endowed with a suitably high π-electron density. It is therefore not surprising that a compound such as thianaphtheno[3,2-*b*]quinoline (XXVI), which lacks both a true L-zone and a K-zone, is devoid of activity.

Degree of correlation with carcinogenicity. From the present study it is clear that there no more exists a *close* correlation between zoxazolamine-hydroxylase inducing effects and carcinogenicity *in situ* in aromatic hydrocarbons, than in the earlier investigated family of polycyclic heterocycles.[5] From the qualitative point of view, a typical carcinogen such as benzo[*c*]phenanthrene can be entirely devoid of inducing activity, and conversely, a non-carcinogen, like dibenz[*a*,*c*]anthracene,[15] can be an extremely potent inducing agent; and, as was already stressed previously,[3] the presence of a reactive L-zone, a molecular feature known to be unfavourable for tumour-producing properties,[12] constantly leads to compounds active as zoxazolamine-hydroxylase inducers. Quantitatively, the methylation of benzo[*a*]pyrene, which increases or at least maintains the carcinogenicity of the basic hydrocarbon, is, on the contrary, detrimental in respect of enzyme-inducing activity. The weak, but statistically significant inducing activity of the four steroid (oestriol) and triterpenoid (asiaticoside, and asiatic and madecassic acid) polyalcohols (none of these substances is carcinogenic *in situ* in the mouse) points in the same direction.

There are however certain features of carcinogenic hydrocarbons that are also present in zoxazolamine-hydroxylase inducing hydrocarbons, and which are indicative of some pattern of molecular action common to both categories of biologically active compounds. There is for instance the striking fact that from among the 33 carcinogens in the present study as many as 25 are active inducers. Also relevant is the fact that electron-accepting substituents always weaken or destroy both types of biological

effect under discussion. As already pointed out with regard to polycyclic enzyme-inducing heterocycles,[5] it is difficult to consider such parallelisms as entirely fortuitous, especially in view of similar observations already made concerning another microsomal hydroxylating enzyme, which converts biphenyl into its 2-hydroxy- derivative.[16] Whether these covariations can be made use of in research on chemical carcinogenesis will be examined in a subsequent paper.

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