Biochemical Pharmacology, Vol. 17, pp. 1227–1236. Pergamon Press. 1968. Printed in Great Britain

THE EFFECT OF BENZOCARBAZOLES AND BENZACRIDINES ON THE PARALYSING ACTION OF ZOXAZOLAMINE; STRUCTURE/ACTIVITY RELATIONSHIPS

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(*Received* 9 *November* 1967; *accepted* 31 *January* 1968)

Abstract—A large number of polycyclic carbazoles and acridines and similar nitrogen-containing heterocycles exhibit a high degree of activity as reducers of zoxazolamine-produced paralysis in young rats, most probably through stimulation of zoxazolamine-hydroxylase synthesis. This activity is not necessarily linked to the presence of carcinogenicity, although certain relationships between structure and activity (e.g., in respect of molecular dimensions, π-electron densities, nature and site of substituents, lipid solubility) are very similar for both types of biological effect.

THE ABILITY of polycyclic carcinogenic hydrocarbons to induce the production of certain oxidative microsomal enzyme systems in the body is of considerable importance for both theoretical and practical reasons, and has therefore been the subject of extensive research in recent years.[1] One such system whose stimulation in young rats can be conveniently and accurately measured *in vivo*, is zoxazolamine hydroxylase, by means of which the paralysing drug 2-amino-5-chlorobenzoxazole is physiologically inactivated by aromatic ring hydroxylation.[2] Inhibition of zoxazolamine-produced paralysis is observed not only with carcinogenic hydrocarbons, such as benzo[*a*]pyrene and dibenz[*a*,*h*]anthracene, but also with non-carcinogens such as naphthacene[3] and many of its derivatives and analogues;[4] strong inhibitory activity has also been reported in both carcinogenic and non-carcinogenic heterocyclic compounds such as dibenzacridines[5] and indophenazines.[6] Although such findings indicate that this action bears no direct correlation with tumour-inducing activity, some striking analogy was observed between the two biological phenomena: in both cases, for instance, the presence of a strong hydrophilic polar substituent (carboxyl, phenolic hydroxyl) greatly diminishes or annihilates the activity—this suggesting the possibility of a common route of transport to the biochemical receptors, if not identity of the biochemical targets themselves. In order therefore, to acquire deeper insight into the similarities and differences between chemical carcinogenesis and stimulation of the synthesis of microsomal enzymes, we undertook an investigation of the effects on zoxazolamine hydroxylase, of as many families as possible of polycyclic compounds known to contain carcinogens and in which relationships between carcinogenicity and structure had already been established. The recent discovery that two standard stimulators of microsomal hydroxylase synthesis, viz. 3-methylcholanthrene and phenobarbital, also stimulate the activity of RNA-polymerase[7] (this suggesting a

direct or indirect influence on gene activity) brings added interest to such an investigation. We report here our studies on benzocarbazoles, benzacridines, and similarly built nitrogen-bearing heterocycles, using the pharmacological test based on the reduction of the duration of zoxazolamine-produced paralysis. The close parallelism found between zoxazolamine hydroxylase levels and duration of paralysis in experiments with hydrocarbons[2, 3] points to the value of using the paralysis test as a means of estimating rises in enzyme levels, although some inactivation of zoxazolamine could conceivably be achieved by non-enzymatic processes (phenylalanine and tryptophan can in this way be hydroxylated[17] to a small extent in the presence of tetrahydropteridine).

MATERIAL AND METHODS

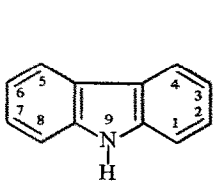
The animals used were 3-month-old male Wistar rats weighing between 90 and 130 g; each animal was used in one experiment only. They were fed for 1 week on a synthetic, vitamin-rich diet,[8] which they continued to receive during the experiment. The substance to be tested was given by i.p. injection (in corn oil solution for all compounds except the insoluble phenarsazines which were given as suspensions in aqueous gum solution; the solvents alone have no effect). A uniform dosage of 20 mg/kg was used, except when the toxicity of the substances necessitated a lower dose; with some substances displaying high activity, additional experiments were made using lower dosages. Control tests were run side-by-side with the experiments, the controls receiving the solvent alone. Twenty-four hours after the injection, both treated animals and controls received an i.p. injection of zoxazolamine at a dose of 90 mg/kg, in corn oil solution. Paralysis occurs between 1 and 2 min after zoxazolamine administration and its duration was estimated by the time (expressed in minutes) taken by the animals to regain their righting reflex. Decrease in the duration of paralysis is assumed to parallel an increase in the level of zoxazolamine hydroxylase.[2] The results were computed statistically by the method of variance analysis and are given in the Tables.

The synthesis of the substances tested has been reported in various publications;[9] many of the compounds have previously been tested for carcinogenic activity.[10]

RESULTS

Table 1 records the results obtained with derivatives of tri- and tetracyclic carbazoles (belonging to basic structures I to V), and in Table 2 are listed the results obtained with dibenzocarbazoles (basic structures VI, VII and VIII), naphthocarbazoles, and carbazoles having more than five rings.

In Table 3 are found heterocyclic compounds which are derived from various benzo- and dibenzocarbazoles by replacing a benzene ring by an equivalent heterocyclic ring such as thiophen, selenophene, thiazole, pyridine, pyrazine, or thiopyran; the close structural kinship of such compounds with their carbazole models is illustrated by comparison of the formulae of 10*H*-thieno[3,2-*a*]carbazole (IX), 12*H*-benzo-[*a*]thieno[2,3-*i*]carbazole (X), and 12*H*-benzo[*g*]thieno[3,2-*a*]carbazole (XI with those of benzo[*a*]carbazole (II), dibenzo[*a*,*i*]carbazole (VI), and dibenzo[*a*,*g*]carbazole (VII), respectively. Table 4 shows results obtained with derivatives of acridine (XII), benz[*a*]acridine (XIII), benz[*c*]acridine (XIV), and more condensed acridines; Table 5 concerns a few compounds derived from condensed acridines by replacing one benzene ring by a thiophen, an α-pyrone, or a pyridine heterocycle, and Table 6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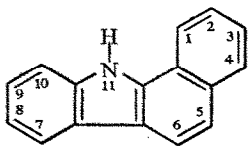
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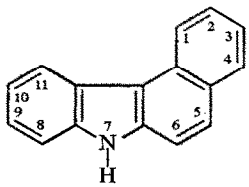
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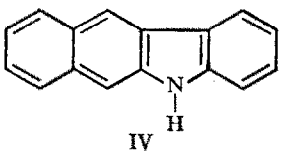
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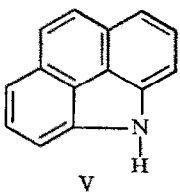
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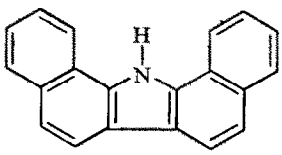
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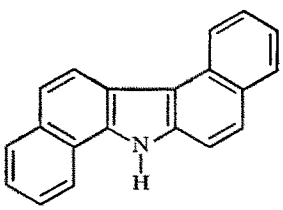
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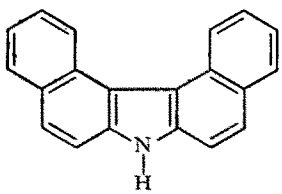
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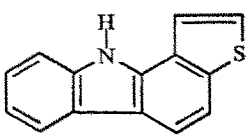
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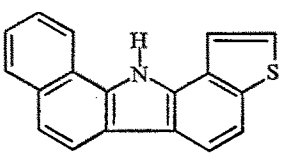
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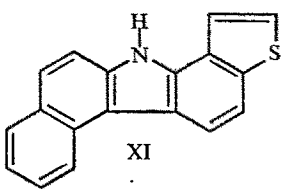
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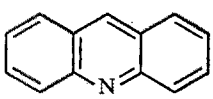
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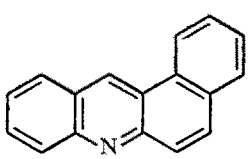
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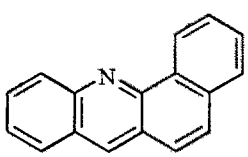

XIV

records results with a series of compounds whose activity or inactivity as enzyme inducers is pertinent to the discussion of structure/activity relationships.

DISCUSSION OF RESULTS

In the following discussions, it is understood that "activity" or "inactivity" relates to a single, 24-hr test time.

The results show that a pronounced reduction of zoxazolamine-induced paralysis is brought about by a great number of nitrogenous heterocyclic compounds belonging to the polycyclic carbazole and acridine families and whose molecules contain from four to six annellated rings.

Carbazole derivatives. Whereas 9-ethylcarbazole is inactive, activity appears with the introduction of an amine group (3-amino-9-ethylcarbazole), and from the 58 remaining substances listed in Tables 1 and 2, all of which have more than three rings and show much diversity in structure and substituents, only eight are devoid of a significant inducing activity, and it is of note that four among these have a very bulky

TABLE 1. CARBAZOLES AND BENZOCARBAZOLES

Substance	Duration of paralysis (min)*	
	Treated	Controls
9-Ethylcarbazole	257 ± 50 (5)	220 ± 93 (10)
3-Amino-9-ethylcarbazole	177 ± 25 (3)	238 ± 60 (5)
2-Chloro-11*H*-benzo[*a*]carbazole	35 ± 10 (6)	338 ± 60 (9)
2-Chloro-11*H*-benzo[*a*]carbazole	28 ± 6 (5)	264 ± 85 (13)
2-Methylthio-11*H*-benzo[*a*]carbazole	69 ± 22 (5)	282 ± 83 (10)
6-Methyl-11*H*-benzo[*a*]carbazole	39 ± 8 (6)	240 ± 73 (10)
8-Methyl-11*H*-benzo[*a*]carbazole	37 ± 8 (7)	334 ± 66 (11)
8-Bromo-11*H*-benzo[*a*]carbazole	35 ± 19 (6)	410 ± 70 (13)
8-*tert*.Amyl-11*H*-benzo[*a*]carbazole	294 ± 92 (5)†	260 ± 82 (10)
8-*tert*.Amyl-11*H*-benzo[*a*]carbazole	259 ± 85 (5)†	262 ± 79 (10)
2-Isopropyl-11*H*-benzo[*a*]carbazole	207 ± 26 (7)†	200 ± 68 (10)
7,10-Dimethyl-11*H*-benzo[*a*]carbazole	99 ± 19 (4)†	200 ± 68 (10)
2-Bromo-8-methyl-11*H*-benzo[*a*]carbazole	28 ± 6 (5)	264 ± 85 (13)
2-Chloro-8-methyl-11*H*-benzo[*a*]carbazole	16 ± 9 (6)	200 ± 68 (10)
2-Methoxy-8-methyl-11*H*-benzo[*a*]carbazole	84 ± 25 (5)	264 ± 85 (13)
2-Nitro-8-methyl-11*H*-benzo[*a*]carbazole	35 ± 14 (6)	343 ± 74 (13)
2,4,7,10-Tetramethyl-11*H*-benzo[*a*]carbazole	176 ± 30 (5)†	260 ± 82 (10)
1,4,7,10-Tetramethyl-11*H*-benzo[*a*]carbazole	120 ± 26 (6)	260 ± 82 (10)
1,2,4,8,10-Pentamethyl-11*H*-benzo[*a*]carbazole	140 ± 32 (5)	238 ± 49 (10)
1,2,4,8,9-Pentamethyl-11*H*-benzo[*a*]carbazole	43 ± 13 (5)	238 ± 49 (10)
2-Bromo-5,6-dihydro-11*H*-benzo[*a*]carbazole	40 ± 8 (5)	325 ± 96 (10)
8-Bromo-2-chloro-5,6-dihydro-11*H*-benzo[*a*]carbazole	35 ± 8 (5)	325 ± 96 (10)
7,8,9,10-Tetrahydro-11*H*-benzo[*a*]carbazole	40 ± 11 (5)	238 ± 49 (10)
5,6-Dihydro-11*H*-benzo[*a*]carbazole	90 ± 25 (2)	282 ± 83 (10)
10-Methyl-7*H*-benzo[*c*]carbazole**	50 ± 10 (5)	272 ± 67 (13)
8,9-Dimethyl-7*H*-benzo[*c*]carbazole	40 ± 13 (5)	238 ± 49 (10)
8,9,10,11-Tetrahydro-7*H*-benzo[*c*]carbazole	167 ± 52 (5)‡	238 ± 49 (10)
5-Acetyl-5*H*-benzo[*b*]carbazole	23 ± 10 (6)	302 ± 61 (10)
6-Nitro-5*H*-benzo[*b*]carbazole	135 ± 15 (3)	367 ± 97 (8)
6-(2,5-Dimethyl-1-pyrryl)-5*H*-benzo[*b*]carbazole	362 ± 85 (4)†	367 ± 97 (8)
4*H*-Benzo[*def*]carbazole	103 ± 23 (6)	250 ± 79 (10)
4-Nitroso-4*H*-benzo[*def*]carbazole	318 ± 59 (5)†	335 ± 26 (8)
1-Nitro-4*H*-benzo[*def*]carbazole	198 ± 48 (5)	335 ± 26 (8)
1-Acetyl-4*H*-benzo[*def*]carbazole	345 ± 32 (5)†	335 ± 26 (8)

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

** Denotes carcinogenicity in this and all the following tables.

† $P > 0.05$.

‡ $0.01 < P < 0.05$.

For all results except those otherwise marked, $P < 0.01$.

structure. Indeed, some of the benzo- and dibenzocarbazoles examined rank among the most active enzyme inducers known. Regarding the nature of the substituents, the presence of a halogen (chlorine, bromine) in the molecule has a favourable effect, whereas that of a nitro or an acetyl group can be deactivating. Electron-donating substituents are favourable provided that they are *not too bulky*, or that they *do not* impart a hydrophilic character to the molecule (deactivating effect of phenolic groups). It is interesting to note that whilst with benzo[*a*]carbazole, substitution with the

bulky isopropyl group is detrimental to activity, it is not so for dibenzo[*a,g*]carbazole, and a possible reason for this significant difference will be discussed later. As exemplified by the 30 substances listed in Table 3, replacement in the molecule of an active polycyclic carbazole, of a benzene ring by a π-isoelectronic one results in still active

TABLE 2. DIBENZOCARBAZOLES, NAPHTHOCARBAZOLES, AND MORE CONDENSED CARBAZOLES

Substance*	Duration of paralysis (min)¶ Treated	Controls
7*H*-Dibenzo[*c,g*]carbazole**	60 ± 20 (7)	272 ± 50 (6)
13*H*-Dibenzo[*a,i*]carbazole**	37 ± 13 (8)	272 ± 50 (6)
2-Chloro-13*H*-dibenzo[*a,i*]carbazole	49 ± 15 (5)	325 ± 96 (10)
2-Methoxy-13*H*-dibenzo[*a,i*]carbazole	55 ± 12 (7)	338 ± 60 (9)
2-Methylthio-13*H*-dibenzo[*a,i*]carbazole	20 ± 10 (6)	200 ± 68 (10)
2-Nitro-13*H*-dibenzo[*a,i*]carbazole	55 ± 25 (6)	335 ± 75 (10)
3-Hydroxy-13*H*-dibenzo[*a,i*]carbazole	105 ± 25 (5)	295 ± 50 (6)
2,3-Dimethoxy-13*H*-dibenzo[*a,i*]carbazole	73 ± 17 (6)	272 ± 67 (13)
3-Chloro-2-hydroxy-13*H*-dibenzo[*a,i*]carbazole	100 ± 31 (5)	264 ± 85 (13)
2,3-Dimethyl-13*H*-dibenzo[*a,i*]carbazole	82 ± 22 (5)	410 ± 70 (13)
9-Chloro-7*H*-dibenzo[*a,g*]carbazole	38 ± 7 (6)	410 ± 70 (13)
9-Chloro-7*H*-dibenzo[*a,g*]carbazole	23 ± 4 (5)	264 ± 85 (13)
9-Bromo-7*H*-dibenzo[*a,g*]carbazole	33 ± 3 (6)	264 ± 85 (13)
9-Methyl-7*H*-dibenzo[*a,g*]carbazole	29 ± 12 (6)	343 ± 74 (13)
9-Methylthio-7*H*-dibenzo[*a,g*]carbazole	36 ± 10 (5)	343 ± 74 (10)
9-Isopropyl-7*H*-dibenzo[*a,g*]carbazole	34 ± 8 (6)	410 ± 70 (13)
13-Methyl-7*H*-dibenzo[*a,g*]carbazole	26 ± 6 (5)	207 ± 35 (12)
9,10-Dimethyl-7*H*-dibenzo[*a,g*]carbazole	27 ± 5 (7)	334 ± 66 (11)
9,10-Dimethyl-7*H*-dibenzo[*a,g*]carbazole	44 ± 9 (5)	410 ± 70 (13)
8,9,11-Trimethyl-7*H*-dibenzo[*a,g*]carbazole	33 ± 10 (5)	238 ± 49 (10)
7,9,10-Trimethyl-7*H*-dibenzo[*a,g*]carbazole	32 ± 2 (5)	264 ± 85 (13)
7,8,11-Trimethyl-7*H*-dibenzo[*a,g*]carbazole	43 ± 6 (4)	264 ± 85 (13)
7,9,11-Trimethyl-7*H*-dibenzo[*a,g*]carbazole	50 ± 10 (3)	260 ± 82 (10)
2,13-Dimethyl-11*H*-naphtho[2,1-*a*]carbazole	360 ± 95 (5)§	238 ± 49 (10)
3-Methyl-13*H*-naphtho[1,2-*a*]carbazole	84 ± 14 (5)	343 ± 74 (13)
13*H*-Acenaphtho[4,5-*a*]benzo[*g*]carbazole	55 ± 10 (5)	274 ± 81 (12)
13*H*-Acenaphtho[4,5-*a*]benzo[*g*]carbazole	67 ± 21 (6)	410 ± 70 (13)
15*H*-Phenanthro[3,2-*a*]carbazole	270 ± 70 (5)†	246 ± 40 (10)
1-Methyl-15*H*-phenanthro[3,2-*a*]carbazole	270 ± 30 (5)	246 ± 40 (10)
15*H*-Benzo[*g*]phenanthro[2,3-*a*]carbazole	233 ± 54 (5)‡	343 ± 74 (13)
16*H*-Triphenyleno[2,3-*a*]carbazole	217 ± 38 (5)	343 ± 74 (13)

* Nomenclature used in this Paper is that of "The Ring Index", Patterson-Capell-Walker, 2nd edn, *Am. Chem. Soc.*, Washington D.C. (1960).
† $P > 0{\cdot}05$.
‡ $0{\cdot}01 < P < 0{\cdot}05$
¶ Compound showing a lengthening effect ($P < 0{\cdot}01$) on the duration of paralysis.
§ Legend for these columns as for Table 1.

compounds (indeed, the 17th compound listed in Table 3 is one of the most active substances found so far), especially when the replacement ring (thiophen, selenophene) does not too greatly alter the behaviour of the molecule with regard to the formation of charge-transfer π-complexes, as a pyridine, and especially a pyrazine, ring often do (witness the weak activity of some of the quinoloindoles in Table 3, compared with the high activity of the parent benzocarbazoles). The lack of activity of various arylindoles and arylbenzindoles (see Table 6), which can be considered as "open" models of active benzo- and dibenzocarbazoles, points probably to the necessity

of having a relatively flat molecule; this is further evidenced by the very high activity displayed by the four benzindeno-indoles investigated (also Table 6), in which the thickness of the molecule is kept to a minimum by the methylene bridge of the cyclopentadiene ring. In this context, it is also highly significant that whereas the *N*-arylnaphthylamines are totally inactive, a flattening of the molecule as achieved by building

TABLE 3. POLYCYCLIC CARBAZOLES IN WHICH A BENZENE RING IS REPLACED BY A HETEROCYCLE

Substance	Duration of paralysis (min)*	
	Treated	Controls
9-Methyl-10*H*-thieno[3,2-*a*]carbazole	32 ± 2 (5)	264 ± 85 (13)
2-Methyl-10*H*-thieno[3,2-*a*]carbazole	47 ± 8 (5)	260 ± 82 (10)
7,10-Dimethyl-10*H*-thieno[3,2-*a*]carbazole	39 ± 7 (5)	272 ± 67 (13)
2,6,9-Trimethyl-10*H*-thieno[3,2-*a*]carbazole	56 ± 20 (6)	212 ± 100 (14)
1,3-Dimethyl-10*H*-thieno[3,4-*a*]carbazole	71 ± 25 (6)	335 ± 73 (10)
1,3,9-Trimethyl-10*H*-thieno[3,4-*a*]carbazole	75 ± 24 (5)	260 ± 82 (10)
1,3,8-Trimethyl-10*H*-thieno[3,4-*a*]carbazole	65 ± 10 (6)	410 ± 70 (13)
8-Methyl-10*H*-[1]benzothieno[3,2-*b*]indole	36 ± 7 (3)	272 ± 67 (13)
7-Methoxy-3-methyl-10*H*-[1]benzothieno[3,2-*b*]indole	54 ± 9 (5)	260 ± 82 (10)
7-Methoxy-2,3-dimethyl-10*H*-[1]benzothieno[3,2-*b*]indole	70 ± 24 (4)	260 ± 82 (10)
2-Methyl-12*H*-benzo[*a*]thieno[2,3-*i*]carbazole	71 ± 25 (6)	338 ± 60 (9)
2-Methyl-12*H*-benzo[*g*]thieno[3,2-*a*]carbazole	61 ± 28 (6)	410 ± 70 (13)
2-Ethyl-12*H*-benzo[*g*]thieno[3,2-*a*]carbazole	40 ± 13 (6)	410 ± 70 (13)
1,3-Dimethyl-12*H*-benzo[*g*]thieno[3,4-*a*]carbazole	55 ± 5 (4)	272 ± 67 (13)
10-Methoxy-7*H*-benzo[*e*]thianaphtheno[3,2-*b*]indole	35 ± 10 (5)	260 ± 82 (10)
6*H*-[1]Benzoselenopheno[3,2-*b*]thiazolo[4,5-*e*]indole	37 ± 6 (5)	240 ± 73 (10)
2-Methyl-6*H*-[1]benzoselenopheno[3,2-*b*]thiazolo[4,5-*e*]indole	16 ± 8 (5)	302 ± 61 (10)
6*H*-[1]Benzoselenopheno[3,2-*b*]thiazolo[5,4-*e*]indole	31 ± 8 (5)	243 ± 76 (10)
8-Methyl-11*H*-quinolo[4,3-*b*]indole	177 ± 44 (5)	246 ± 40 (10)
8,9-Dimethyl-11*H*-quinolo[4,3-*b*]indole	190 ± 59 (5)†	246 ± 40 (10)
13*H*-Benzo[*g*]quinolo[4,3-*b*]indole	139 ± 47 (5)	246 ± 40 (10)
13*H*-Benzo[*e*]quinolo[4,3-*b*]indole	41 ± 15 (5)	246 ± 40 (10)
13*H*-Benzo[*g*]pyrido[3,2-*a*]carbazole**	58 ± 6 (5)	410 ± 70 (13)
6,11-Dimethyl-13*H*-benzo[*a*]pyrido[3,2-*i*]carbazole	126 ± 52 (6)	343 ± 74 (13)
10-Methoxy-12*H*-pyrido[3,2-*g*]thianaphtheno[3,2-*b*]indole	73 ± 15 (5)	260 ± 82 (10)
10-Methoxy-7*H*-pyrido[3,2-*e*]thianaphtheno[3,2-*b*]indole	42 ± 8 (5)	260 ± 82 (10)
9,11-Dichloro-8*H*-benzo[*f*]indolo[2,3-*b*]quinoxaline	51 ± 9 (5)	242 ± 18 (8)
9,11-Dimethyl-8*H*-benzo[*f*]indolo[2,3-*b*]quinoxaline	104 ± 45 (4)	242 ± 18 (8)
9-Methyl-12-chloro-8*H*-benzo[*f*]indolos[2,3-*b*]quinoxaline	136 ± 26 (5)	242 ± 18 (8)
5,10-Dihydrothieno[2′,3′:2,3]thiopyrano[4,3-*b*]indole	95 ± 30 (5)	339 ± 102 (10)

* Legend for these columns as for Table 1.
† $0{\cdot}01 < P < 0{\cdot}05$.

a bridge between the aryl and the naphthyl group by means of an —AcCl—group (7-chloro-7,12-dihydrobenzo[*c*]phenarsazine) results, again, in very active compounds (Table 6).

Acridine derivatives. From Table 4 it can be seen that the enzyme inducing activity of the benzacridines is generally lower than that found among the benzocarbazoles (although, as reported in an earlier study,[5] the *di*benzacridines are at least as potent as the *di*benzocarbazoles, if not more so). The rules governing structure/activity relationships are, however, the same: for example, introduction of a hydroxy group removes all activity and a bulky substituent like a phenyl one drastically reduces it, while halogen substitution leads to highly active compounds in both the benz[*a*]acridine and the benz[*c*]acridine series. Table 5, which lists results obtained with

TABLE 4. ACRIDINES, BENZACRIDINES, AND MORE CONDENSED ACRIDINES

Substance	Duration of paralysis (min)¶	
	Treated	Controls
3-Methylacridine	121 ± 47 (5)†	250 ± 79 (10)
4-Fluoro-8,10-dimethylacridine	170 ± 35 (6)†	207 ± 35 (12)
Benz[*a*]acridine	95 ± 15 (6)	320 ± 90 (13)
8-Fluorobenz[*a*]acridine	57 ± 7 (5)	194 ± 51 (10)
9-Fluorobenz[*a*]acridine	62 ± 10 (5)	194 ± 51 (10)
10-Fluorobenz[*a*]acridine	34 ± 5 (5)	194 ± 51 (10)
4-Hydroxybenz[*a*]acridine	310 ± 46 (5)†	274 ± 81 (12)
10-Methylbenz[*a*]acridine	60 ± 20 (6)	349 ± 55 (10)
12-Methylbenz[*a*]acridine	154 ± 28 (5)	349 ± 55 (10)
10-Aminobenz[*a*]acridine**	186 ± 43 (5)	282 ± 83 (10)
10-Methoxy-12-methylbenz[*a*]acridine	74 ± 6 (5)	274 ± 81 (12)
10-Hydroxy-2-*tert*.amylbenz[*a*]acri*d*ine	322 ± 47 (6)*	207 ± 35 (12)
10,12-Dimethylbenz[*a*]acridine	47 ± 18 (6)	335 ± 73 (10)
8-Fluoro-12-phenylbenz[*a*]acridine	163 ± 40 (6)†	194 ± 51 (10)
9-Chloro-12-ethyl-8-methylbenz[*a*]acridine	152 ± 18 (5)	274 ± 81 (12)
3,8,9,12-Tetramethylbenz[*a*]acridine	170 ± 45 (6)	320 ± 90 (13)
9-Trifluoromethyl-12-propylbenz[*a*]acridine	261 ± 47 (5)	410 ± 70 (13)
Benz[*c*]acridine	156 ± 19 (6)	349 ± 55 (10)
9-Fluorobenz[*c*]acridine	30 ± 9 (5)	349 ± 55 (10)
7-Methylbenz[*c*]acridine**	171 ± 27 (6)	349 ± 55 (10)
9-Methylbenz[*c*]acridine	33 ± 10 (5)	349 ± 55 (10)
7-Methyl-9-phenylbenz[*c*]acridine**	145 ± 35 (7)	320 ± 90 (13)
7-Ethyl-10-trifluoromethylbenz[*c*]acridine	75 ± 22 (5)	295 ± 50 (6)
Tribenz[*a,c,h*]acridine	62 ± 2 (2)	282 ± 83 (10)
Benz[*a*]naphtho[1,2-*j*]acridine	173 ± 46 (6)†	200 ± 68 (10)
10*H*-Benz[*c*]indeno[1,2-*h*]acridine	110 ± 25 (5)	207 ± 35 (12)
8*H*-Benz[*a*]indeno[1,2-*h*]acridine	52 ± 17 (6)	256 ± 80 (10)
1-Methylacenaphtho[1,2-*b*]quinoline§	21 ± 5 (6)	410 ± 70 (13)
4-Nitroquinoline-*N*-oxide**	330 ± 47 (5)†	335 ± 73 (10)

* Compound showing a lengthening effect ($P < 0{\cdot}01$) in the duration of paralysis.

† $P > 0{\cdot}05$.

‡ Substance included here for similarity of its molecular form (presence of a tetracyclic-linear complex) with benz[*b*]acridine.

§ This simple carcinogenic quinoline compound is included here as relevant to the discussion of relationships between carcinogenicity and enzyme induction.

¶ Legend for these columns as for Table 1.

TABLE 5. CONDENSED ACRIDINES IN WHICH ONE BENZENE RING IS REPLACED BY A HETEROCYCLE

Substance	Duration of paralysis (min)‡	
	Treated	Controls
8-Methylthiaquindoline	146 ± 30 (5)	343 ± 74 (13)
6-Ethylthiaquindoline	265 ± 52 (5)*	343 ± 74 (13)
1-Methylthieno[3,2-*a*]benz[*h*]acridine	27 ± 6 (5)	349 ± 55 (10)
Pyrido[3,2-*f*]thianaphtheno[3,2-*b*]quinoline	87 ± 21 (5)	240 ± 73 (10)
10*H*-Indeno[1,2-*h*]coumarino[4,3-*b*]quinoline	410 ± 92 (5)†	240 ± 73 (10)

* $P > 0{\cdot}05$.

† Compound showing a lengthening effect ($P < 0{\cdot}01$) on the duration of paralysis.

‡ Legend for these columns as Table 1.

TABLE 6. ARYLINDOLES, INDENOINDOLES, AND OTHER HETEROCYCLES STRUCTURALLY AKIN TO BENZOCARBAZOLES

Substance	Duration of paralysis (min)	
	Treated	Controls
3-Phenyl-2-(2-thienyl)indole	297 ± 16 (5)†	243 ± 60 (10)
1-Methyl-2-phenyl-3*H*-benz[*e*]indole	280 ± 25 (3)†	243 ± 60 (10)
1-Phenyl-2-(2,3-dimethyl-3-thienyl)-3*H*-benz[*e*]indole	194 ± 57 (5)†	191 ± 67 (14)
10-Methyl-7,12-dihydrobenz[*g*]indeno[1,2-*b*]indole	34 ± 9 (6)	410 ± 70 (13)
10-Ethyl-7,12-dihydrobenz[*g*]indeno[1,2-*b*]indole	30 ± 13 (6)	191 ± 67 (14)
7,12-Dihydrobenz[*e*]indeno[1,2-*b*]indole**	25 ± 11 (6)	410 ± 70 (13)
9-Ethyl-7,12-dihydrobenz[*e*]indeno[1,2-*b*]indole	19 ± 9 (6)	200 ± 68 (10)
2-(4-Fluoro-3-methylphenyl)indolizine	172 ± 33 (5)	325 ± 96 (10)
2-(4-Biphenylyl)imidazo[1,2-*c*]pyrimidine	166 ± 40 (5)	325 ± 96 (10)
trans-1-(10-Methyl-3-phenothiazinyl)-2-phenylethylene	320 ± 71 (5)*	234 ± 30 (10)
7-Chloro-7,12-dihydrobenzo[*c*]phenarsazine (at 5 mg/kg)	55 ± 14 (6)	339 ± 102 (10)
7-Chloro-7,12-dihydrobenzo[*c*]phenarsazine (at 2·5 mg/kg)	55 ± 14 (6)	339 ± 102 (10)
6-Chloro-6,11-dihydro[1,4]benzarsazino[3,2-*b*]thianaphthen (at 5 mg/kg)	178 ± 44 (4)‡	320 ± 80 (10)
6-Chloro-6,11-dihydro[1,4]benzarsazino[3,2-*b*]thianaphthen (at 1 mg/kg)	280 ± 56 (4)†	320 ± 80 (10)
12-Chloro-7,12-dihydro-2,3,9,10-tetramethyl-benzo[*a*]phenarsazine (at 5 mg/kg)	293 ± 47 (5)†	320 ± 80 (10)
17-Chloro-8,17-dihydrodinaphtho[2,3-*a*:2′,3′-*j*]phenarsazine	321 ± 72 (5)†	323 ± 100 (10)
N-Phenyl-α-naphthylamine§	300 ± 74 (5)†	335 ± 26 (8)
N,*N*′-β,β′-Dinaphthylamine§	305 ± 78 (5)†	335 ± 26 (8)

* Compound tested also at dose of 40 mg/kg, and showing a highly significant lengthening effect ($P < 0.01$) on paralysis at this dose.

† $P > 0.05$.

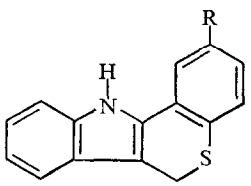
‡ Two animals out of 6 died at this dose.

§ These compounds, non-heterocyclic, are included because they could be considered as "open" models of 11*H*-benzo[*a*]carbazole and 7*H*-dibenzo[*c*,*g*] carbazole respectively.

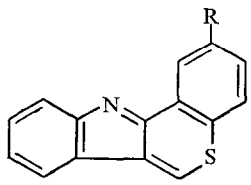
five compounds derived theoretically from benzacridines or more condensed acridines by replacing one benzene ring by a heterocycle (thiophen, α-pyrone), shows that, depending on the site of this replacement, there is maintenance of a high activity (3rd compound listed), or sharp decrease in activity (2nd compound), or even, paradoxically, a lengthening effect on the duration of paralysis (5th compound) which is statistically highly significant.

Zoxazolamine hydroxylase synthesis induction and carcinogenicity. The present study confirms the lack of direct correlation already reported in earlier papers for other types of compounds,[3–6] between carcinogenicity and this particular type of enzyme-inducing activity, since the great majority of the compounds active as inducers are non-carcinogenic. It should be noted, however, that with the sole exception of 4-nitroquinoline-*N*-oxide, all of the compounds reported here which had already proven carcinogenic,[10] are also powerful enzyme inducers in the present test; what is more, this one exception concerns a tumour-producing agent which in the strict sense cannot be classified among the polycyclic carcinogens in view of the simplicity of its structure and its high chemical activity. It is unlikely that this coexistence of biological effects is fortuitous, considering that the relationships which have emerged between structure and activity bear so close a resemblance to those found to exist in carcinogenicity. The most important molecular feature of polycyclic carcinogens, as it is

well known,[11, 12] is the presence of a dense network of conjugated aromatic double bonds, certain areas of which (e.g. *meso*anthracenic region or L-zone, *meso*phenanthrenic region or K-zone) are endowed with high π-electron densities; a reduction of this network by suppresion of one or several of the double bonds decreases the carcinogenicity of the molecule.[13] That this is likewise so in the case of enzyme induction is demonstrated by the hydrogenated carbazoles of Table 1, and also by the two pairs of [1]benzothiopyrano[4,3-*b*]indoles (XV and XVII, XVI and XVIII) recently reported[14]: as seen from Table 7, each hydrogenated member of the pair is significantly



XV; R=Cl
XVI; R=CH_3



XVII; R=Cl
XVIII; R=CH_3

less active than its completely aromatic counterpart ($P < 0{\cdot}01$).

TABLE 7. COMPARATIVE ENZYME-INDUCING ACTIVITIES

Compound	Duration of paralysis (min)*
XV	117 ± 29 (5)
XVII	71 ± 7 (5)
XVI	129 ± 20 (5)
XVIII	68 ± 7 (5)

* Legend for this column as for Table 1.

The importance of K-zones in polycyclic carcinogens also has its parallel among the enzyme inducers investigated here: just as the isosteric replacement in the carcinogenic benz[*c*]acridines, of the K-zone by a sulphur heteroatom destroys the tumour-producing activity,[15] so does the same replacement considerably diminish the enzyme-inducing activity, as comparison of 9-methylbenz[*c*]acridine (Table 4) with 8-methylthiaquindoline (Table 5) shows. It is perhaps relevant to note here that inactivation by substitution with a bulky substituent is achieved more easily in benzo[*a*]carbazoles which have only one K-zone (9th compound listed in Table 1) than in dibenzo[*a*,*g*]-carbazoles, which have two (15th compound, Table 2).

Creaven *et al.*,[16] investigating the stimulating effects of carcinogenic and non-carcinogenic hydrocarbons on an enzymatic system that hydroxylates biphenyl at position 2, found a distinct parallelism between enzyme-stimulation and carcinogenicity. A study, on the lines of the present work, of the effects of carcinogenic and non-carcinogenic hydrocarbons in regard to zoxazolamine hydroxylase synthesis will shortly be reported from our Laboratory.

CONCLUSION

Apart from the phenarsazines which proved toxic, and those carbazoles and acridines which are carcinogenic, the active inducers reported here showed no apparent

sign of other pharmacological activities (e.g. on the central nervous system). And since it is likely that the substrate specificity of the microsomal enzyme system called "zoxazolamine hydroxylase" is not limited just to zoxazolamine but extends to other substrates with aromatic rings, the high degree of efficacy of certain nitrogen heterocyclics as stimulators of the *in vivo* synthesis of this particular enzyme suggests that they might profitably replace agents such as methylcholanthrene or phenobarbital in pharmacological or clinical efforts to combat deficiencies in enzymatic hydroxylation. There is also the possibility that these same heterocyclic inducers might, like methylcholanthrene, stimulate other enzymatic systems (for instance, RNA-polymerase) of more fundamental importance for molecular biology; this possibility warrants closer investigation.

Acknowledgement—The authors thank Laboratoires Laroche Navarron (92-Levallois, France) for a grant to one of us (D.-P.H.) and for providing facilities for this work, and the Régie Nationale Française des Tabacs (Director of Research, Mr. P. Vespérini) for financial support.

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