THE GROWTH-INHIBITORY AND CARCINOGENIC PROPERTIES OF 4-AMINOSTILBENE AND DERIVATIVES

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Marked inhibition of the growth of the Walker rat carcinoma 256 is produced by administration of 4-aminostilbene and of 4-dimethylaminostilbene. From similar experiments with the Crocker sarcoma 180, the carcinoma C63, and spontaneous mammary cancer, it appears that the growth-inhibitory action of these compounds is much less pronounced in mice.

In a dosage of 200 to 250 mg./kg. in the rat, both 4-amino- and 4-dimethylaminostilbene produce (*a*) gastro-intestinal submucous haemorrhage most evident in the pyloric portion of the stomach, (*b*) haematuria, and (*c*) haemolymph changes, while the former compound induces methaemoglobinaemia in addition.

Using the Walker rat carcinoma 256 as the biological test object, a series of derivatives of 4-aminostilbene has been examined to determine the relationship between the growth-inhibitory effect and chemical constitution.

The great majority of the active compounds can be defined as stilbenes with a basic substituent, the position of which is of paramount importance; thus *o*-dimethylaminostilbene is very much less active than the *p*-isomeride, and the *m*-compound is completely inactive. A further essential feature is the ethylene bridge, since activity disappears when either of its hydrogen atoms is substituted, when the bridge is reduced, when it is extended to contain three or four carbon atoms, or when either methine group is replaced by a nitrogen atom. Compounds in which the ethylene bridge is absent, or is replaced by oxygen or sulphur, are also inactive, and activity is further dependent upon the *trans* configuration of the molecule about the ethylenic bond, and to a large extent upon a free *p'*-position.

These and other facts have suggested the working hypothesis that one of the features required for activity is an unbroken conjugation of the amino group with both nuclei, enabling the compound to assume some dipolar quinonoid character, which depends, among other things, on the co-planar arrangement of the two benzene nuclei which characterizes the *trans* form of the stilbenes.

When evidence concerning steric conditions in the molecules (obtained mainly from the ultra-violet spectroscopy of 4-dimethylaminostilbene and thirteen of its alkyl derivatives) is compared with the biological activities of these compounds, a close parallel is suggested between lack of growth-inhibitory power and buckling of the molecule. Thus in the 4-dimethylaminostilbene derivatives with substituents on the α- and β-carbon atoms of the ethylenic double bond, or with methyl groups at two *ortho* positions in a phenyl group, steric factors reduce the planarity of the molecule, thus affecting the conjugation resonance characteristic of the whole molecule. These compounds had previously been found to be non-inhibitory. All the evidence from diagrams, models and spectra suggests that steric interference with the planar configuration of molecules in this series varies continuously from the planar 4-dimethylaminostilbene to the highly buckled $\alpha\beta$-diethyl derivative. Inhibitory activity within this series appears to depend upon a conjunction of such factors as molecular size and shape, and the apposition of a planar molecule to a hypothetical adsorbing surface.

In view of the previously suggested connexion between growth-inhibitory activity and tumour production, a number of selected aminostilbenes has been tested for carcinogenicity. Of seventy-two rats exposed to the action of 4-amino-, 4-acetamido-, 4-dimethylamino-, or 4-diethylamino-stilbene, twenty-three developed a total of eight sarcomata and thirty distant tumours mostly comprising squamous keratinizing carcinomata of the external acoustic duct, mammary adenomata, and cholangiomata. In a second series, the compounds 4-amino-, 4-dimethylamino- and 2′-methyl-4-dimethylaminostilbene, and 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene, were tested in male and female mice and rats. Of 120 rats exposed, forty-eight developed a total of twenty sarcomata at the site of injection and fifty-one tumours in organs distant from the site of injection, while of 120 mice similarly treated only four sarcomata and two distant tumours were recorded in five. The nature and distribution of the distant tumours induced in the rat strongly suggest some common feature in the carcinogenic action of aminostilbenes and 2-acetamidofluorene.

Other points discussed include (*a*) the association between haemolymphatic changes and carcinogenesis, (*b*) the possible significance of the nitrogenous analogue 2-(4′-dimethylaminostyryl)quinoline in relation to the carcinogenic action of 'styryl 430', (*c*) a comparison of the biological properties of 4-dimethylaminostilbene and 4-dimethylaminoazobenzene, and (*d*) the dependence for manifestation of the growth-inhibiting action of the aminostilbenes upon a sufficiently low intake of dietary protein.

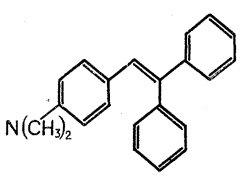
I. Introduction

Earlier papers (Haddow 1935, 1938*a*; Haddow & Robinson 1937, 1939; Haddow & Scott 1937; Badger, Elson, Haddow, Hewett & Robinson 1942) have described a growth-inhibitory property, affecting tissues both normal and malignant, as an associated biological feature in the carcinogenic hydrocarbons. This association has always been construed as possessing etiological meaning, tumour production being conceived as an adaptive reaction to a highly characteristic or even specific interference with the growth of normal cells. The evidence supporting such an interpretation, or consistent with it, has been fully reviewed elsewhere (Haddow 1937, 1938*b*, 1947).

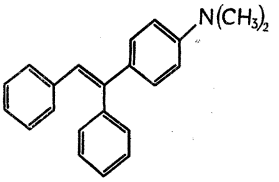
Among the chemical types studied by Badger *et al.* (1942), various derivatives of the oestrogen triphenylethylene (Robson & Schönberg 1937) were examined. Some of these compounds were found to produce a mild retarding effect on the growth of the Walker rat carcinoma 256, and triphenylchloroethylene and triphenylmethylethylene in particular were later studied by Watkinson, Delory, King & Haddow (1944) for their action upon cancer of the prostate in man, and by Haddow, Watkinson & Paterson (1944) for their influence upon advanced malignant disease in general.

In continuation of this work, compounds with a basic substituent in one or in two of the phenyl rings of the triphenylethylene or related systems were examined, e.g.

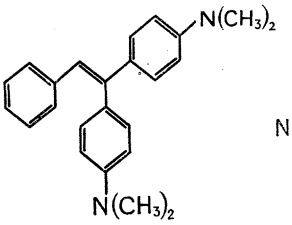
1:1-diphenyl-2-(4′-dimethylaminophenyl)ethylene (I), 1:2-diphenyl-1-(4′-dimethylaminophenyl)ethylene (II), 2-phenyl-1:1-di(4′-dimethylaminophenyl)ethylene (III) and 9-(4′-dimethylaminobenzylidene)fluorene (IV):



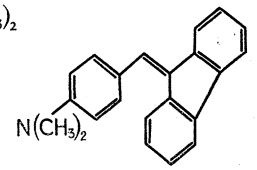
I



II

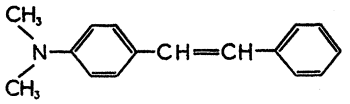


III



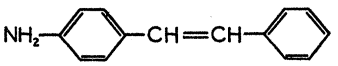
IV

Of these, only (IV) had any activity, but it was decided to test the action of 4-dimethylaminostilbene (V), the skeleton of which is contained in all the above compounds:



V

At once it became obvious that this compound produced an unusually marked interference in the growth of the Walker tumour, and the same property was found to be present in 4-aminostilbene (VI): the inhibitory effect was, however, much less manifest in mice (in experiments with the Crocker sarcoma 180, the carcinoma C63, and spontaneous mammary cancer (Haddow, Harris & Kon 1945)).



VI

A further point of interest is that none of the aminostilbenes employed appears to possess oestrogenic activity; in this connexion it may be recalled that Baker (1943) examined an amino analogue of hexoestrol (3:4-*bis*-(*p*-aminophenyl)hexane), and reported it to have no oestrogenic activity in either the *meso* or the racemic form.

The inhibitory action of 4-aminostilbene and of 4-dimethylaminostilbene on the growth of the Walker tumour was demonstrable only within limits, on account of the toxicity of both these substances. In one experiment in which twenty-three rats of about 100 g. weight were implanted with this carcinoma, and twelve of them given an injection of 5 mg. of 4-dimethylaminostilbene (dissolved in 2 c.c. of arachis oil) intraperitoneally on the following day and again 1 week later, the remainder being treated with arachis oil alone, the individual tumour weights (g.) on removal on the 14th day are shown in table 1.

In another experiment in which eleven tumour-implanted rats were similarly treated with 5 mg. of 4-dimethylaminostilbene intraperitoneally followed by three doses of the same amount subcutaneously at intervals of a few days, and compared with eleven control

animals, the tumour weights at the 11th day totalled 262·4 g. (mean 23·8 g.) in the latter series, against a total of only 4·0 g. in the former. In a third experiment, twelve implanted rats received 5 mg. of 4-dimethylaminostilbene intraperitoneally followed by four subcutaneous injections of the same amount; in a comparison with twelve control tumours at the 13th day, the latter showed a total tumour weight of 354·3 g. (mean 29·5 g.), while the whole treated series produced a total of only 4·7 g. Similar results were observed for 4-aminostilbene; in one experiment in which a dose of 20 mg. was administered intraperitoneally, the tumours in the control series averaged 24·8 g. as against 2·9 g. in the treated series (ratio 8·55, $n=25$, $t=2·39$, $P=0·02$), while in another experiment in which the dose was only 5 mg., the corresponding values were 25·4 and 6·8 g. (ratio 3·74, $n=18$, $t=2·92$, $P<0·01$). The main cytological effect in these experiments has been identified by Dr P. C. Koller as chromosome fragmentation in the tumour cells.

TABLE 1

control series	series treated with 4-dimethylaminostilbene
61·8 55·5 40·0 38·7 31·9 25·7	33·7 19·2 17·2 8·8 7·0 6·1
22·3 17·9 11·3 10·2 5·6	5·6 0·0 0·0 0·0 0·0 0·0
mean: 29·2 g.	mean: 8·1 g.

ratio of means: 3·60. $P<0·01$

The toxic effects observed after administration of both these substances in a dosage of 200 to 250 mg./kg. were first a pronounced degree of gastro-intestinal submucous haemorrhage largely confined to the pyloric portion of the stomach, and secondly haematuria. The former effect had not been encountered previously, and seems from other experiments to be a characteristic property of certain derivatives of 4-aminostilbene; its mechanism is not known, although the appearances are suggestive of a concentrated secretion or excretion of the compound (or of a metabolic product) restricted to the region of the pylorus. Further, in the case of 4-aminostilbene (but not of 4-dimethylaminostilbene), methaemoglobinaemia was apparent in the 24 hr. following a single intraperitoneal injection of 200 mg./kg. This observation was checked by Dr E. Boyland, and is of interest in relation to the studies of Lester, Greenberg & Shukovsky (1944) of methaemoglobin formation in the rat by β-phenylhydroxylamine and p-aminophenol (intermediary products of the metabolism of aniline). Only one further instance of methaemoglobin formation was observed during the present experiments, in the case of 2-chloro-4-aminostilbene (*infra*); here again the property was absent in the corresponding 4-dimethylamino compound.

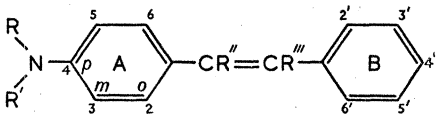
Another toxic manifestation, of special interest in the sequel, was the causation by 4-aminostilbene and 4-dimethylaminostilbene of haemolymph changes. Such changes were also produced by related compounds tested subsequently, of which the most active in this respect are noted in the tables which follow. While such activity is a feature of many derivatives of 4-aminostilbene, it is clearly not exclusive to this class of compound, since it is identical with that described by Lasnitzki & Woodhouse (1942, 1944) in rats treated with the carcinogens 1:2:5:6-dibenzanthracene, 3:4-benzpyrene and 20-methylcholanthrene. Since Lasnitzki & Woodhouse failed to observe haemolymph changes with anthracene and phenanthrene, they suggested that the ability of a polycyclic hydrocarbon to produce such alterations might in some way be connected with its carcinogenic action;

the present results lend support to this suggestion, and its possible significance will be referred to later.

In view of these effects a series of derivatives of 4-aminostilbene was systematically studied. The majority of the compounds was tested on the Walker rat carcinoma 256. This material has been used merely in the sense of a growing test object judged useful by experience, and there would obviously be advantages in conducting experiments of this kind with a series of such test objects, of varying biological reactivity. The Walker carcinoma has, however, been chosen first for its relative resistance to minor interference, and secondly because it is not so refractory as not to show a response to agents such as the synthetic triphenylethylene oestrogens (*supra*) and urethane (Haddow & Sexton 1946), the action of both of which was detected by its use. Any major chemical interference with the growth of this tumour is therefore likely to have biological significance, and to be worthy of further attention. The method of assay of the inhibitory effect was the standard experiment previously described (Badger *et al.* 1942, p. 257), the compound under test being administered (usually intraperitoneally in solution or suspension in sterile arachis oil) to an experimental series of rats on the day following implantation with the Walker carcinoma, and the effect, if any, being gauged by comparison of the tumour weights with those of a control series similarly implanted and given the solvent alone, after an interval of between 2 and 3 weeks. In the following tables details are given of each experiment, the complete data being summarized by the values of C and T (the mean weights (g.) of the control and treated tumours respectively), the ratio C/T, and of n, t and P in the t test.

II. Influence upon growth-inhibitory action of modifications in chemical constitution

Systematic modifications of the structure represented by VII were examined, such modifications being designed to affect one prominent feature at a time, e.g. the polar group, the ethylene bridge, and rings A and B.



VII

(1) *Modifications involving the polar group*

(i) *The* ortho-, meta- *and* para-*isomerides*

From table 2 it appears that 2-dimethylaminostilbene, while falling far short of the inhibitory activity of 4-dimethylaminostilbene, still retains it to a slight extent, the effect in the experiment quoted being of high significance. On the other hand, 3-dimethylaminostilbene appears to have no trace of such activity; the possible implications of this contrast are referred to below.

Table 2

experiment number	compound	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) C	mean tumour weights (g.) T	C/T	n	t	P
1	3-dimethylaminostilbene	200	14	21·5	28·7	0·749	20	1·50	0·2
2	2-dimethylaminostilbene	200	11	21·7	12·2	1·78	21	3·34	<0·01

(ii) *Compounds involving substitution in the amino group, and other derivatives*

The compounds in which the amino group of 4-aminostilbene is unsymmetrically substituted, with a single methyl or ethyl group, still exhibit activity, while the toxicity of these compounds is not greatly less than that of 4-aminostilbene itself. That their inhibitory action is, however, probably less than in 4-aminostilbene or 4-dimethylaminostilbene is shown in table 3, which should be compared with the data for the two former compounds at equivalent dosage. Similarly, a loss of activity is indicated in 4-diethylaminostilbene as against 4-dimethylaminostilbene. While these compounds are practically indistinguishable at sufficiently high dosage, whether in their inhibitory action, general toxicity or in the production of special effects such as submucous haemorrhage or haemolymph changes, all these properties appear to be manifested at lower concentrations of the dimethyl than of the diethyl compound; it will, however, be noted that marked inhibitory activity was shown by an asymmetric acetoxyethyl derivative of 4-diethylaminostilbene, and high activity by 4-acetamidostilbene. Even at considerable dosage, little or no effect was observed with 4-diallylaminostilbene. Three azostilbenes were examined, both on general grounds and on the supposition that they might act as precursors of 4-aminostilbene by fission of the azo linkage; no inhibitory effects were, however, observed. A result of special importance is the failure to demonstrate any action in an aminostilbene quaternary salt, at any rate under the conditions employed; in this case, of course, the resonance of the amino group with the stilbene residue is destroyed.

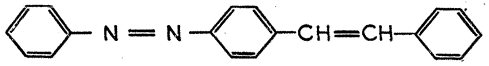
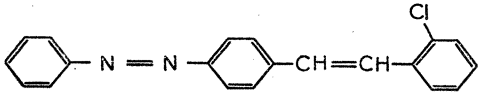
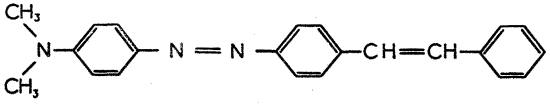
(iii) *Replacement of the polar group*

A number of substances was examined to determine whether activity might still be retained in compounds in which the amino group was replaced (table 4), but apart from an extremely slight effect in the case of stilbene-4-sulphonamide, no statistically significant inhibition was observed. Special interest attaches to the negative result obtained with 4-*iso*propylstilbene, as indicating the non-interchangeability of the amino and *iso*propyl groups in spite of their similarity as electron donors, and with the two benzylamines tested (see Kon 1948). The lack of activity in 4-amidinostilbene is of similar interest, as also in relation to the effects of stilbamidine (4:4′-diamidinostilbene) upon myeloma, recently described by Snapper & Schneid (1946); in another series of experiments, no action upon the growth of the Walker tumour was detected as a result of treatment with stilbamidine or its 2-amino-, 2-chloro- or 2-iodo-derivatives.

2. *Modifications of the ethylene bridge*

Thirty-five experiments were performed, and are summarized in table 5, to test the activity of compounds in which the ethylene bridge had been modified (*a*) by extension so as to include three or four carbon atoms (experiments 22 to 25), (*b*) by alkyl substitution of the ethylene hydrogens (experiments 26 to 32), (*c*) by oxidation or reduction, (*d*) by total removal (experiments 47 to 55), (*e*) by other alteration or replacement, e.g. the replacement of one bridge methine by nitrogen (experiments 38, 39), or of the entire bridge by oxygen or sulphur. Apart from a transient inhibition produced by 4-aminotolane, which might be attributable to reduction to 4-aminostilbene *in vivo*, it is remarkable that only two experiments (numbers 26 and 35) yielded any positive result of statistical significance, and that

TABLE 3

experiment number	compound	*R*	*R′*	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) *C*	*T*	*C*/*T*	*n*	*t*	*P*
3	4-methylaminostilbene	H	CH_3	50	14	10·5	4·2	2·5	18	1·37	0·2
4	4-acetamidostilbene*	H	CH_3CO	100	14	20·2	0·7	**28·9**	17	8·88	<0·01
5	4-ethylaminostilbene	H	C_2H_5	50	14	10·5	4·0	2·62	22	1·65	0·1
6	4-diethylaminostilbene	C_2H_5	C_2H_5	50	15	18·0	8·9	2·02	20	1·54	0·1
7	4-*N*-(ethyl-*β*-acetoxyethyl)-aminostilbene	C_2H_5	$C_2H_4OCOCH_3$	200	15	22·9	2·1	**10·9**	11	5·47	<0·01
8	4-diallylaminostilbene	CH_2:CH.CH_2	CH_2:CH.CH_2	200	17	29·8	20·8	1·4	17	1·36	0·2
9	4-dimethylaminostilbene methosulphate			7 × 10	16	34·7	35·2	0·98	17	0·08	0·94
10	4-phenylazostilbene			200	17	41·5	39·6	1·05	14	0·30	0·8
11	2′-chloro-4-phenylazostilbene			200	17	41·5	43·0	0·96	13	0·2	0·8
12	4-(4′-dimethylaminophenyl)-azostilbene			200	17	41·5	51·0	0·81	13	1·62	0·1

* Produced submucous haemorrhage in pylorus.

TABLE 4

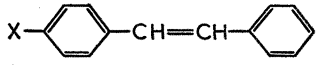
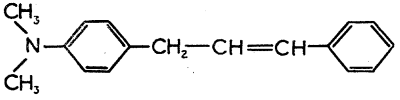
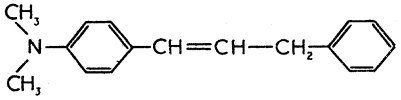
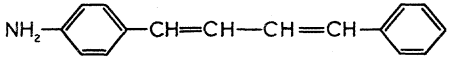
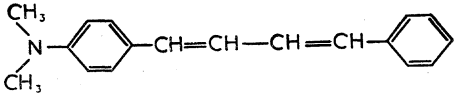
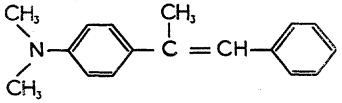
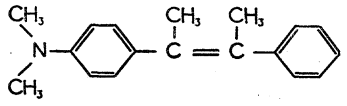
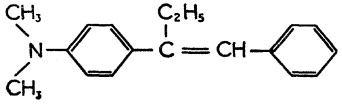
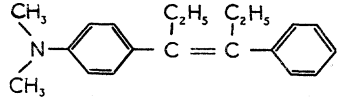
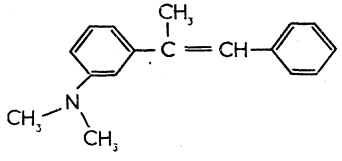
experiment number	compound 	—*X*	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) *C*	*T*	*C*/*T*	*n*	*t*	*P*
13	4-methylstilbene	—CH_3	200	14	9·7	12·2	0·79	16	0·43	0·7
14	4-*iso*propylstilbene	—CH.$(CH_3)_2$	200	16	49·1	40·5	1·2	18	1·23	0·2
15	4-nitrostilbene	—NO_2	200	15	25·4	15·7	1·62	20	1·39	0·2
16	4-cyanostilbene	—CN	200	20	29·2	48·5	0·60	9	1·23	0·3
17	4-amidinostilbene	—C(:NH)NH_2	180	16	49·1	42·8	1·15	15	0·92	0·4
18	4-methylthiostilbene	—S.CH_3	200	16	33·6	12·8	2·62	11	1·49	0·2
19	stilbene-4-sulphonamide	—SO_2NH_2	200	14	17·0	12·0	1·42	22	2·02	0·05
20	*N*-dimethyl-4-styrylbenzylamine	—$CH_2N(CH_3)_2$	200	18	49·9	44·9	1·11	9	1·04	0·3
21	4-styrylbenzylamine	—CH_2NH_2	180	13	32·1	33·1	0·97	18	0·39	0·7

TABLE 5

experiment number	compound	formula	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) C	mean tumour weights (g.) T	C/T	n	t	P
22	1-phenyl-3-(4′-dimethylaminophenyl)prop-1-ene		200	16	31·8	27·4	1·16	21	0·72	0·5
23	3-phenyl-1-(4′-dimethylaminophenyl)prop-1-ene		60	15	29·8	29·8	1·0	20	0·0	1·0
24	4′-amino-1:4-diphenylbutadiene		200	14	30·0	26·4	1·14	22	0·54	0·6
25	4′-dimethylamino-1:4-diphenylbutadiene		200	14	41·1	44·4	0·926	20	0·50	0·6
26	4-dimethylamino-α-methylstilbene		200	12	29·2	20·6	1·42	22	2·73	0·01
27	4-dimethylamino-αβ-dimethylstilbene		500	14	23·4	29·5	0·79	21	1·64	0·1
28	4-dimethylamino-α-ethylstilbene		500	14	23·4	25·3	0·925	21	0·51	0·6
29	4-dimethylamino-αβ-diethylstilbene*		200	15	24·4	23·4	1·04	20	0·19	0·9
30	3-dimethylamino-α-methylstilbene		200	14	27·8	23·7	1·17	21	0·48	0·6

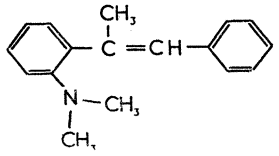
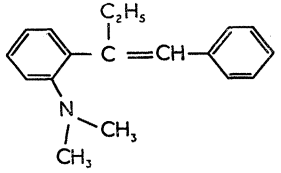
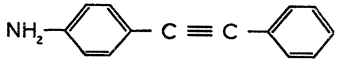
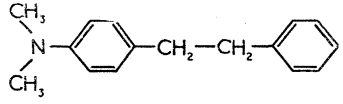
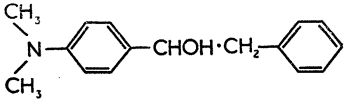
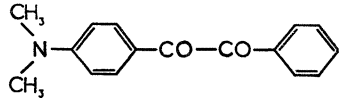
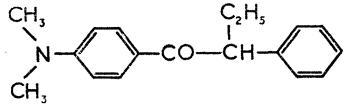
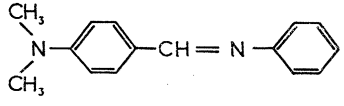
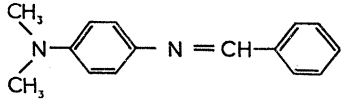
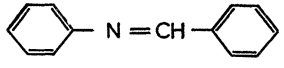
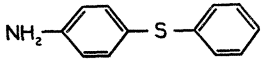
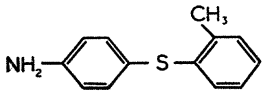
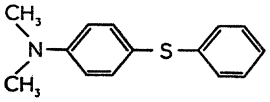
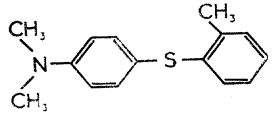
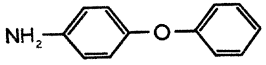
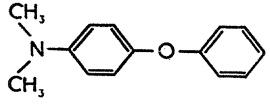
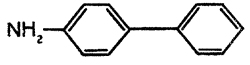
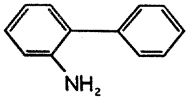
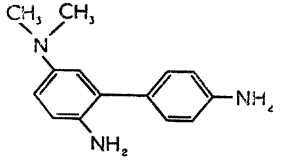
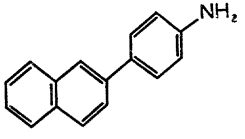
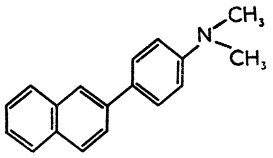
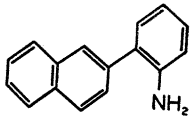
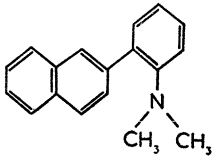
31	2-dimethylamino-α-methylstilbene		200	11	21·7	19·7	1·10	21	0·63	0·5
32	2-dimethylamino-α-ethylstilbene		500	16	38·4	46·4	0·828	18	1·29	0·2
33	4-aminodiphenyl-acetylene (4-aminotolane)		180	24	21·6	12·2	1·77	13	0·85	0·4
34	4′-dimethylamino-1:2-diphenylethane		200	17	22·7	35·0	0·65	21	1·58	0·1
35	1-phenyl-2-(4′-dimethyl-aminophenyl)ethan-2-ol		200	19	29·2	15·2	1·97	20	2·64	0·02
36	4-dimethylaminobenzil		200	17	22·9	36·2	0·633	21	2·02	0·05
37	4-dimethylamino-α-ethyl-deoxybenzoin		200	15	29·8	32·8	0·91	17	0·61	0·6
38	4-dimethylamino-benzylideneaniline		200	21	52·6	43·8	1·20	19	0·76	0·5
39	4′-dimethylamino-benzylideneaniline		200	17	28·6	36·4	0·78	16	0·54	0·6
40	benzylideneaniline		200	19	27·7	31·1	0·89	21	0·34	0·7
41	4-aminodiphenylsulphide		200	17	27·7	16·5	1·68	18	1·23	0·2

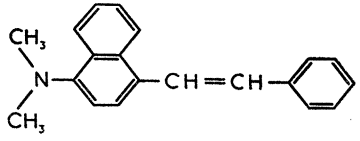
TABLE 5 (*cont.*)

experiment number	compound	formula	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) *C*	*T*	*C*/*T*	*n*	*t*	*P*
42	4-amino-2′-methyl diphenylsulphide		200	13	19·3	28·8	0·67	17	1·11	0·3
43	4-dimethylamino-diphenylsulphide		180	14	47·6	52·6	0·90	15	0·89	0·4
44	4-dimethylamino-2′-methyl diphenylsulphide		200	14	46·8	49·0	0·95	13	0·35	0·7
45	4-aminodiphenylether		180	11	32·5	36·0	0·90	15	0·88	0·4
46	4-dimethylamino-diphenylether		200 180	17 11	58·7 32·5	63·4 33·8	0·93 0·96	14 15	0·74 0·32	0·5 0·8
47	4-aminodiphenyl (*p*-xenylamine)		200	15	38·6	36·7	1·05	10	0·23	0·8
48	4-dimethylaminodiphenyl (*NN*-dimethyl-*p*-xenylamine)		100	14	13·8	16·3	0·847	20	0·57	0·6
49	2-aminodiphenyl (*o*-xenylamine)		200	17	22·9	29·6	0·774	20	0·93	0·4
50	2-dimethylaminodiphenyl (*NN*-dimethyl-*o*-xenylamine)		200	14	13·8	17·3	0·798	20	0·75	0·5
51	2:4′-diamino-5-dimethylaminodiphenyl		20	19	31·9	37·5	0·85	15	0·51	0·6
52	2-(4′-aminophenyl)-naphthalene		200	16	40·2	28·4	1·41	13	0·89	0·4

53	2-(4′-dimethylamino-phenyl)naphthalene		180	14	47·6	43·6	1·09	16	0·69	0·5
54	2-(2′-aminophenyl)-naphthalene		200	13	19·3	18·1	1·07	16	0·14	0·9
55	2-(2′-dimethylamino-phenyl)naphthalene		200	17	27·7	23·5	1·18	18	0·44	0·7

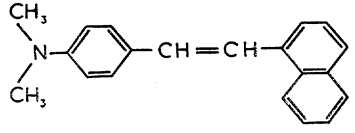
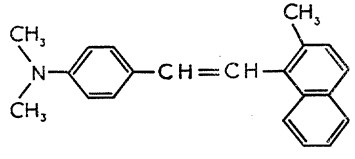
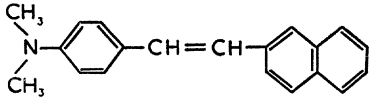
* 4-Amino-$\alpha\beta$-diethylstilbene was examined by Brownlee, Copp, Duffin & Tonkin (1943) in a study of the antibacterial properties of various amino-, cyano-, amidino- and nitro- derivatives of stilbene.

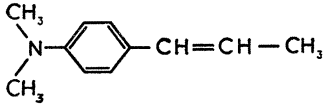
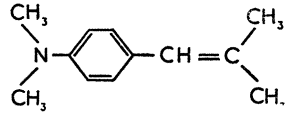
TABLE 6

experiment number	compound	formula	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) *C*	mean tumour weights (g.) *T*	*C*/*T*	*n*	*t*	*P*
56	2-phenyl-1-(4′-dimethyl-amino-1′-naphthyl)-ethylene		200	18	28·0	18·0	1·56	17	0·94	0·3
57	2-chloro-4-aminostilbene*		180	19	22·7	24·7	0·919	15	0·22	0·8
58	2-chloro-4-dimethyl-aminostilbene†		200	19	22·7	31·8	0·714	13	1·09	0·3
59	2:4-diaminostilbene‡		200	15	17·6	4·9	3·59	22	2·96	$<0·01$
				14	41·1	16·5	2·49	19	5·99	$<0·01$
60	2-amino-4-acetamido-stilbene		100	18	42·5	23·5	1·81	19	3·35	$<0·01$
61	4-amino-2-acetamido-stilbene		100	18	42·5	17·4	2·44	14	3·40	$<0·01$
62	2-amino-4-methylamino-stilbene		100	16	19·5	16·8	1·16	17	0·33	0·7

* Produced methaemoglobinaemia. † No methaemoglobinaemia. ‡ Hypnotic.

TABLE 7

experiment number	compound	formula	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) C	mean tumour weights (g.) T	C/T	n	t	P
A. Alkyl and dialkyl derivatives and related compounds										
63	4-amino-2′-methylstilbene*		180	15	24·9	3·3	**7·54**	16	4·16	<0·01
64	4-dimethylamino-2′-methylstilbene*		180	13	20·0	1·0	**20·0**	22	5·09	<0·01
65	4-dimethylamino-2′-ethylstilbene		200	20	27·6	12·3	2·24	16	2·19	0·05
66	4-dimethylamino-2′-*n*-propylstilbene		200	15	32·6	33·9	0·962	15	0·26	0·8
67	4-diethylamino-2′-methylstilbene		200	16	39·4	28·2	1·397	13	1·10	0·3
68	4-amino-4′-methylstilbene		200	17	22·7	28·0	0·811	19	0·59	0·6
69	4-dimethylamino-4′-methylstilbene		200	16	38·4	41·9	0·916	17	0·61	0·5
70	4-dimethylamino-2′:3′-dimethylstilbene		200	19	32·8	10·7	3·07	17	1·96	0·05
71	4-dimethylamino-2′:4′-dimethylstilbene		200	16	39·4	51·5	0·765	14	2·10	0·05
72	4-dimethylamino-2′:5′-dimethylstilbene		200	18	22·7	2·4	**9·46**	16	4·30	<0·01
73	4-dimethylamino-2′:5′-diethylstilbene		200	21	32·0	28·6	1·12	12	0·25	0·8
74	4-dimethylamino-2′:5′-dipropylstilbene		200	18	22·7	17·5	1·30	16	1·00	0·3
75	4-dimethylamino-2′:5′-di*iso*propylstilbene		200	21	32·0	34·7	0·922	13	0·17	0·9
76	4-dimethylamino-2′:5′-di*tert.*butylstilbene		200	20	27·6	28·7	0·962	15	0·10	0·92
77	4-dimethylamino-2′-methyl-5′-*iso*propylstilbene		200	14	29·2	25·0	1·168	16	0·80	0·4
78	4-dimethylamino-2′:6′-dimethylstilbene		180	17	42·5	25·8	1·647	13	2·3	0·05
79	4-dimethylamino-3′:4′-dimethylstilbene		180	17	42·5	26·4	1·61	14	2·15	0·05
80	4-dimethylamino-3′:5′-dimethylstilbene*		180	15	24·9	1·2	**20·75**	11	3·21	<0·01
81	4-dimethylamino-2:2′-dimethylstilbene		200	14	9·7	2·7	3·59	15	1·88	0·1
82	1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene		(*a*) 250§ (*b*) 200†	26 15	4·0 44·7	4·0 1·1	1·0 **40·6**	15 16	0·0 13·38	1·0 <0·01
83	1-(4′-dimethylaminophenyl)-2-(2′-methyl-1′-naphthyl)-ethylene		200	15	44·7	16·2	2·76	16	6·34	<0·01
84	1-(4-dimethylaminophenyl)-2-(2′-naphthyl)ethylene		(*a*) 250§ (*b*) 200	26 15	4·0 44·7	5·0 29·9	0·8 1·49	15 15	0·87 2·87	0·4 0·01

B.	Halogen derivatives								
85	2′-chloro-4-dimethylaminostilbene	150	11	11·6	0·6	**19·3**	17	7·62	<0·01
86	3′-chloro-4-aminostilbene*	180	17	37·6	3·4	**11·059**	14	5·98	<0·01
87	3′-chloro-4-dimethylaminostilbene	180	15	41·8	4·8	**8·71**	17	7·93	<0·01
88	4′-chloro-4-dimethylaminostilbene	180	15	41·8	39·2	1·066	18	0·49	0·6
89	2′-bromo-4-dimethylaminostilbene	200	17	29·8	32·6	0·914	18	0·42	0·7
90	3′-bromo-4-dimethylaminostilbene*	180	17	37·6	1·5	**25·067**	18	8·46	<0·01
91	4′-bromo-4-dimethylaminostilbene	200	25	22·9	34·6	0·662	13	0·84	0·4
92	2′-fluoro-4-dimethylaminostilbene	40	12	35·0	13·9	2·518	17	3·70	<0·01
C.	Oxygen derivatives								
93	4-amino-4′-hydroxystilbene	40	15	38·6	34·5	1·12	18	0·74	0·5
94	4-amino-4′-methoxystilbene‡	200	15	26·1	22·4	1·17	22	0·73	0·5
D.	Nitrogen derivatives								
95	2′:4-diaminostilbene	180	15	41·8	43·5	0·961	17	0·28	0·8
96	2′:4-tetramethyldiaminostilbene	180	15	40·2	40·4	0·995	17	0·03	0·98
97	4-amino-3′-dimethylaminostilbene	180	17	39·8	5·2	**7·654**	16	6·28	<0·01
98	4′-nitro-4-dimethylaminostilbene	180	14	40·9	38·2	1·071	16	0·33	0·7
99	4′-amino-4-dimethylaminostilbene	180	14	23·7	15·6	1·52	20	2·77	0·01
E.	Removal of ring B								
100	4-dimethylamino-β-methylstyrene 	180	19	22·0	52·6	0·418	15	4·90	<0·01
101	4-dimethylamino-$\beta\beta$-dimethylstyrene 	180	16	33·6	38·9	0·864	14	0·50	0·6
102	4-dimethylaminobenzoic acid	180	14	29·2	32·5	0·898	16	0·63	0·5

* Submucous haemorrhage in pylorus. † Haemolymph changes marked. ‡ Produced ophthalmia. § Experiment with mouse carcinoma C63.

even in these cases the effect itself was small and certainly much short of that due to 4-amino- or 4-dimethylaminostilbene. It therefore appears that all such changes either annul activity or reduce it considerably, and, conversely, that maximum inhibition is in some way bound up with the integrity of the ethylene bridge.

3. *Substitution affecting ring* A

Only a few compounds were examined with substituent groups affecting ring A alone, and from table 6 it would seem that there is considerable loss of activity in the 1-naphthyl homologue of 4-dimethylaminostilbene, and in the 2-chloro-derivatives of both 4-amino- and 4-dimethylaminostilbene. On the other hand, activity was still present (although greatly reduced as compared with 4-aminostilbene) in 2:4-diaminostilbene, which compound was of some individual interest as showing pronounced hypnotic properties not encountered in any other member of this series. Slight activity was seen in the 2- and 4-acetamido-derivatives of 4- and 2-aminostilbene, and very marked activity (and toxicity) have since been observed for 2:4-diacetamidostilbene.

4. *Substitution affecting ring* B

Compounds possessing substituents in ring B were more fully examined, and table 7 summarizes the results obtained with alkyl, halogen, oxygen and nitrogen derivatives; from these there have emerged certain conclusions of very considerable interest. First, activity of the same order as in 4-aminostilbene and 4-dimethylaminostilbene, but with somewhat reduced toxicity, was shown by the 2′-methyl derivatives of both these compounds, while the effect was only just detectable in 4-dimethylamino-2′-ethylstilbene and had disappeared in the 2′-*n*-propyl compound (experiments 63 to 66). A similar rule is indicated for the series of 2′:5′-dialkyl derivatives in experiments 72 to 76, activity in this case being restricted to the dimethyl member and absent in those with more bulky substituents. Activity was also noted in 4-dimethylamino-3′-methylstilbene, associated in this case with extreme toxicity, and was also present in the 2′:3′- and 3′:5′-dimethyl derivatives, and possibly to a very slight extent in the 2′:6′-dimethyl compound. A specially interesting contrast is provided by the 2(α)- and 2(β)-naphthyl analogues of dimethylaminostilbene, the individual tumour weights (g.) in experiments 82*b* and 84*b* being as shown in table 8.

TABLE 8

control

64·1 52·0 47·4 45·8 41·9 37·8 37·4 31·0 (mean 44·7 g.)

1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene (experiment 82*b*)

4·9 2·7 0·9 0·7 0·5 0·5 0·2 0·1 0·1 0·0 (mean 1·1 g.)

1-(4′-dimethylaminophenyl)-2-(2′-naphthyl)ethylene (experiment 84*b*)

42·5 41·9 39·0 34·4 33·0 26·6 23·8 14·8 13·5 (mean 29·9 g.)

Both compounds produced a statistically significant inhibition, but, as is evident, this was intense for the α-naphthyl compound and extremely slight for its β-naphthyl isomeride. Also, haemolymph changes were produced by the former and not by the latter compound. These substances provide a striking example of the species difference already referred to, since neither (in experiments 82*a* and 84*a*) affected the growth of the mouse carcinoma C63.

Among the halogen derivatives tested, marked activity was noted for 2′- and 3′-chloro compounds (experiments 85 to 87), and toxicity in these cases appeared to be rather less than for the corresponding methyl analogues. While 2′-chloro- and 3′-chloro-4-dimethylaminostilbene were roughly similar in their effects, a remarkable contrast was seen in the inactivity of the 2′-bromo derivative and the high activity manifested by 3′-bromo-4-dimethylaminostilbene (cf. detailed tumour weights in table 9); this may conceivably be attributed to some physico-chemical factor, still to be ascertained (see below).

TABLE 9

control

52·3 45·0 41·7 36·1 34·0 27·8 26·7 15·2 12·7 7·0 (mean 29·8 g.)

2′-bromo-4-dimethylaminostilbene (experiment 89)

54·5 46·6 43·8 37·5 35·4 34·1 30·0 22·8 18·2 3·6 (mean 32·6 g.)

control

55·8 49·8 49·7 42·2 39·9 39·3 38·0 26·0 18·3 16·5 (mean 37·6 g.)

3′-bromo-4-dimethylaminostilbene (experiment 90)

4·9 3·6 2·6 1·8 1·7 0·3 0·0 0·0 0·0 0·0 (mean 1·5 g.)

Perhaps the most striking single conclusion from the results in table 7 is the virtual disappearance of biological action with the introduction of a 4′-substituent. For ten compounds (experiments 68, 69, 71, 79, 88, 91, 93, 94, 98, 99), representing 4′-methyl-, 2′:4′- and 3′:4′-dimethyl-, 4′-chloro-, -bromo-, -hydroxy-, -methoxy-,* -nitro- and -amino-substitution, negative results were obtained in all with the exception of slight activity in the case of 3′:4′-dimethyl- and 4′-amino-4-dimethylaminostilbene (experiments 79, 99). This important effect, of which an illustration is given in table 10, may possibly be a factor in the relative inactivity of 1-(4′-dimethylaminophenyl)-2-(2′-naphthyl)ethylene as compared with its 2-(1′-naphthyl) isomeride (above); the inactivity of 2′:4-diamino- and 2′: 4-tetramethyldiaminostilbene, in comparison with, say, 4-amino-3′-dimethylaminostilbene (experiments 95, 96, 97), may perhaps be explained by regarding the former two compounds as 2-aminostilbenes with a blocked 4-position in the second ring.

TABLE 10

control

59·2 57·9 53·8 46·1 43·5 35·0 35·0 30·7 29·7 27·0 (mean 41·8 g.)

3′-chloro-4-dimethylaminostilbene (experiment 87)

19·6 15·8 2·7 2·6 2·0 0·4 0·2 0·0 0·0 (mean 4·8 g.)

4′-chloro-4-dimethylaminostilbene (experiment 88)

54·2 50·4 46·0 44·3 40·6 40·0 39·2 38·2 20·7 18·7 (mean 39·2 g.)

Apart from the above, three compounds were tested in which ring B is absent, viz. two dimethylaminostyrenes and 4-dimethylaminobenzoic acid, the last as a conceivable although unproved metabolite of dimethylaminostilbene (experiments 100 to 102); with none of these compounds was any inhibition observed.

* 4-Amino-4′-methoxystilbene was observed to have a curious action in producing marked ophthalmia, the exact nature of which has still to be determined.

5. *Stereoisomerism*

While all the compounds mentioned were of the *trans* configuration about the bridge double bond, it was possible to test a few selected *cis* isomerides in addition (table 11, experiments 103 to 105). All the results were negative, and while that obtained for *cis* 2′:4-diaminostilbene is of less interest on account of the inactivity of the corresponding *trans* form (table 7, experiment 95), a complete contrast is apparent between the inactive *cis* 2′-chloro-4-amino- and -4-dimethylaminostilbenes on the one hand, and their markedly active *trans* isomerides on the other (table 7, experiment 85). Although the cases examined have been few, these examples are suggestive of an essential dependence for biological activity upon the *trans* configuration. Of possibly related interest is the inactivity of 3-acetamidophenanthrene (experiment 107), which could be regarded as a stilbene-like structure in its *cis* form.

TABLE 11

experiment number	compound	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.)	
				C	*T*
103	*cis* 2′:4-diaminostilbene	200	17	29·8	42·5
104	*cis* 2′-chloro-4-aminostilbene	180	15	40·2	40·1
105	*cis* 2′-chloro-4-dimethylaminostilbene	180	15	40·2	35·6
106	2-acetamidophenanthrene	180	15	31·6	25·1
107	3-acetamidophenanthrene	180	15	31·6	31·3
		C/*T*	*n*	*t*	*P*
103	*cis* 2′:4-diaminostilbene	0·701	16	1·67	0·1
104	*cis* 2′-chloro-4-aminostilbene	1·002	17	0·02	0·98
105	*cis* 2′-chloro-4-dimethylaminostilbene	1·129	16	0·70	0·5
106	2-acetamidophenanthrene	1·259	13	1·10	0·3
107	3-acetamidophenanthrene	1·010	14	0·05	0·96

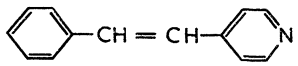
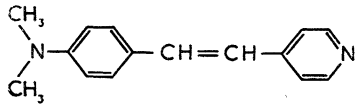
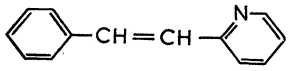
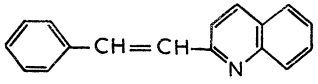
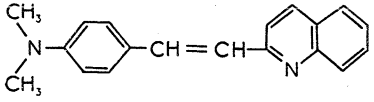
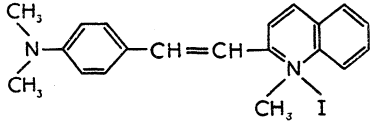
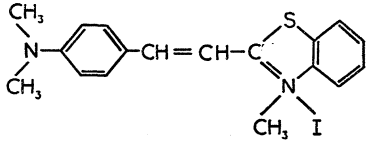
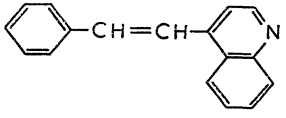
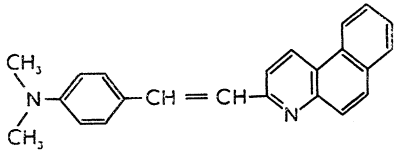
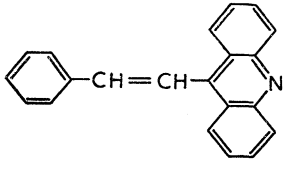
6. *Heterocyclic modifications*

Results obtained with heterocyclic analogues are listed in table 12, and show several features of interest. For example, it is clear (as might be anticipated) that compounds in which a nitrogen atom is contained within the ring system (e.g. α- and γ-stilbazoles, experiments 110, 108), are devoid of the inhibitory properties which may be conferred by the nitrogen atom when part of a basic substituent. It has, however, become equally clear that many nitrogenous analogues of the aminostilbenes may show the same characteristic biological properties, i.e. when they too possess a nitrogen-containing polar group in a suitable position. Thus while 2-styrylquinoline proved inactive, distinct evidence of inhibitory activity was detected for 2-(4′-dimethylaminostyryl)quinoline (experiments 111, 112), although the effect was rather transient and the compound itself markedly toxic under the conditions employed; styrylquinolines of this structure are, of course, suggestive in relation to the carcinogenicity of 'styryl 430' (Browning, Gulbransen & Niven 1936) and this point is further dealt with in the general discussion below. Slight but significant activity was also noted in 5-(4′-dimethylaminostyryl)acridine, in contrast with 5-styrylacridine itself. Further nitrogenous compounds of the pyrimidine and purine type are still under examination.

7. *Conclusions*

It becomes increasingly clear, especially perhaps from the advances in antibacterial chemotherapy of recent years, and other attempts to relate chemical constitution and biological action, that the pharmacodynamics of a given compound—that is, its behaviour in the

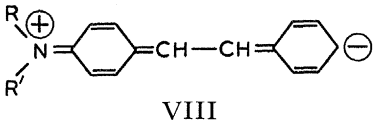
TABLE 12

experiment number	compound	formula	dose (mg./kg.)	duration of experiment (days)	mean tumour weights (g.) C	mean tumour weights (g.) T	C/T	n	t	P
108	4-styrylpyridine (γ-stilbazole)		4 × 200	19	42·1	44·3	0·950	17	0·23	0·8
109	4-(4′-dimethylaminostyryl)-pyridine (4′-dimethylamino-γ-stilbazole)		7 × 10	16	34·7	44·8	0·775	15	1·40	0·2
110	2-styrylpyridine (α-stilbazole)		200	16	46·8	47·8	0·979	17	0·24	0·8
111	2-styrylquinoline		5 × 200	21	24·8	22·6	1·10	13	0·23	0·8
112	2-(4′-dimethylamino-styryl)quinoline		180	24	21·6	6·6	3·27	10	1·51	0·2
113	2-(4′-dimethylaminostyryl)-quinoline methiodide ('styryl 15')		7 × 10	16	34·7	39·9	0·870	17	0·66	0·5
114	2-(4′-dimethylamino-styryl)benzthiazole methiodide		7 × 10	16	34·7	27·9	1·24	16	0·80	0·4
115	2-styryl-6-acetamido-quinoline		200	16	46·5	41·4	1·123	15	0·56	0·6
116	4-styrylquinoline		200	10	11·3	15·9	0·711	17	1·77	0·1
117	2-(4′-dimethylaminostyryl)-5:6-benzoquinoline		200	18	30·3	39·8	0·76	17	1·05	0·3
118	5-styrylacridine		200	10	11·3	13·7	0·825	17	1·21	0·2
119	5-(4′-dimethylamino-styryl)acridine		200	10	11·3	5·0	2·26	17	5·32	0·01

face of numerous absorptive, detoxicating and excretory mechanisms, and its capacity to reach or penetrate the susceptible cell—is as important as its intrinsic or specific biological activity, and is also highly dependent upon the details of chemical structure. It is furthermore true that the biological potency of such a compound may depend particularly upon physico-chemical factors, such as relative solubility in different cell constituents, ability to permeate cell membranes, possible ionization under different physiological conditions, and other properties influenced by the shape and size of the molecule and polar conditions within it; it is hoped in due course to pay adequate attention to all these factors. However, at a fairly early stage in the present investigation it appeared that the aminostilbene series might offer distinct advantages, and that its study, even if largely restricted to the end-result or inhibitory effect alone, might nevertheless yield a coherent picture of the relation, within this class, of constitution and activity. As will be seen, this expectation appears to be realized.

In the first place, the great majority of the active compounds can be defined as stilbenes with a basic substituent, of the general type VII already described (p. 151), and of which 4-aminostilbene is a typical example. It is apparent that while 4-methylamino-, 4-ethylamino-, and particularly 4-dimethylaminostilbenes, are highly active, 4-diethylaminostilbene appears less so and 4-diallylaminostilbene is inactive, while stilbenes in which the 4-amino group is replaced by methyl, *iso*propyl or methylthiol groups are inactive. The position of the basic substituent would seem of paramount importance; thus 2-dimethylaminostilbene is much less active than the 4-isomeride, and the 3-compound is inactive. A further essential feature is the ethylene bridge; activity disappears when either of its hydrogen atoms is substituted, when the bridge is reduced with formation of a diphenylethane derivative, when it is extended to contain three or four carbon atoms as in the isomeric propylenes or the corresponding butadiene, or when either methine group is replaced by a nitrogen atom. Compounds in which the ethylene bridge is absent (diphenyl and phenylnaphthalene derivatives), or is replaced by oxygen or sulphur, have proved inactive, and activity is also dependent upon the *trans* configuration of the molecule about the ethylenic bond, and to a large extent on a free 4-position in ring B.

These and other facts (e.g. that increase in size of substituents in position 2′ leads to diminution of activity) have suggested the working hypothesis that one of the features required for biological effectiveness is an unbroken conjugation of the amino group with both nuclei; according to the modern concepts of resonance dipolar quinonoid structures



VIII

such as (VIII) are indeed canonical structures contributing to the aminostilbene molecule (see below). The contribution of VIII depends, among other things, on the coplanar arrangement of the two benzene nuclei which characterizes the *trans* form of the stilbenes; in the *cis* stereoisomerides the two rings are not coplanar, and the resonance energy is greatly reduced.

That the presence of a second *para* substituent (in position 4′) may entail complete loss of activity, to some extent independent of the nature of the substituent group, is one of the

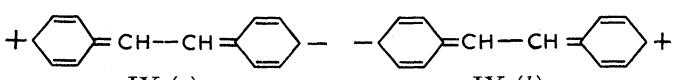
most interesting features so far disclosed. An alkyl substituent in this position would of course tend to suppress the contribution of quinonoid structures (neglecting, in the first instance, the effect of hyperconjugation) by reducing the tolerance of the 4′ carbon atom for a negative charge. This consideration cannot, however, apply to several other groups which produce the same effect, and it appears likely that this phenomenon is bound up with the nature of the metabolism which compounds of this class may undergo in the mammalian body. There is already some evidence to show that they are in part excreted in the form of their 4′-hydroxy derivatives (private communication from Dr L. A. Elson), a result hardly to be expected when another substituent is already present in the required position. Assuming that hydroxylation, i.e. biological oxidation of the 4′ carbon atom, occurs by an electrophilic reaction, it should be favoured by factors causing an increased electron density at that point, and on the whole this is supported by the observed effects of substituents introduced into the stilbene molecule. The comparison already recorded between 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene and the corresponding 2′-naphthyl compound (table 8), is of special interest for the question of 4′-substitution. The latter compound has no free 4′-position in the strict sense, but it is well known that in naphthalene compounds the activation of an α-position can be relayed to other atoms in the adjoining ring. From this point of view it is therefore noteworthy that the 2′-naphthyl compound does in fact possess slight activity, although this is of course far short of that exhibited by the 1′-isomeride. So far as concerns the biological activity of halogen derivatives, a remarkable general similarity was noted between 2′-chloro- and 2′-methyl-4-dimethylaminostilbenes; while the inductive effects of chlorine and methyl substituents are opposed, it is recognized that the introduction of these groups often confers similar pharmacological properties, owing no doubt to their comparable volume (cf. Pauling 1946). The abrupt disappearance of activity observed on passing from the 2′-chloro- to the 2′-bromo- compound is at present unexplained; it is not apparent in the 3′-position, since 3′-bromo-4-dimethylaminostilbene is highly active (see table 7).*

The assumptions regarding the structural features required for the characteristic biological behaviour of the stilbenes lend themselves to examination by physical methods. In the following section an account is given of such an examination by means of ultra-violet spectroscopy.

III. The ultra-violet absorption spectroscopy of selected stilbenes

8. *General theoretical considerations*

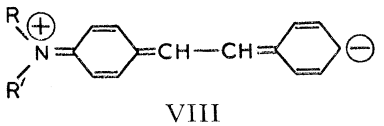
The structures of the stereoisomeric stilbenes have been discussed in terms of resonance theory by Pauling & Sherman (1933) and Pauling (1942) and by Lewis, Calvin, and co-workers (1939, 1940) and Branch & Calvin (1941). Earlier work on their ultra-violet absorption spectra, together with that of *trans* 4-dimethylaminostilbene, has been summarized by Branch & Calvin (1941), who consider that in stilbene the structures important for absorption due to conjugation resonance are polar quinonoid structures such as IX(*a*) and (*b*), with positive



IX (*a*) IX (*b*)

* We have not yet succeeded in preparing the corresponding iodo-derivatives in a pure state.

or negative charge on any of the *ortho* or *para* carbon atoms. In the 4-dimethylamino-derivative there will be an increased contribution from the corresponding optically important structures such as VIII.*



VIII

It is clear that in the latter compound stereochemical factors which distort the benzene rings or the amino group out of the plane of the molecule will reduce the contribution of quinonoid structures, and diminish resonance stabilization due to these structures. Absorption due to conjugation resonance, and characteristic of the whole molecule, should then appear with diminished intensity or at higher frequency (shorter wave-length) or both.†

Examples of these effects have been noted previously in the stereoisomeric stilbenes. Thus, the non-planar *cis* stilbene absorbs at shorter wave-lengths (*c.* 15 mμ for the long wave-length band) than *trans* stilbene (Smakula & Wassermann 1931; Lewis & Calvin 1939), and substitution of one or both α- and β-carbon atoms in *trans* stilbene produces similar changes (Ley & Rinke 1923; Ley & Specker 1939; Arends 1931; and others). There is a reduction in maximum extinction for the long wave-length absorption band in each of these non-planar molecules. Similar results are seen in a series of unsymmetrical 4-dimethylaminostyryl dyes recently examined by Brooker, White, Sprague, Dent & van Zandt (1947), and among less closely related series, in the work on substituted diphenyls summarized by Branch & Calvin (1941) and in other aromatic molecules examined by Jones (1941, 1945).

9. *Technique and results*

The technique of measurement used in this investigation has been described elsewhere (Mayneord & Roe 1935). Ethyl alcohol was used as solvent throughout, and preliminary examinations were made using a hydrogen discharge tube as light source, in order to detect very weak or very narrow bands which may be overlooked by the usual procedure using a spark source of light.

* [*Note added in proof.*] A communication (unpublished) from Professor C. A. Coulson and Miss J. Jacobs, of the calculated π-electron distribution and bond orders in the ground state and the first excited state of the *trans* 4-aminostilbene molecule shows that the contribution of *o*- and *p*-quinonoid structures is very small in the ground state, excess π-electron charge being considerable on the β-carbon atom of the ethylenic double bond and negligible in the unsubstituted benzene ring B. In the first excited state, *o*- and *p*-quinonoid structures make a greater contribution but the highest π-electron charge occurs on the α-ethylenic carbon atom. Recently published calculations by A. Pullman (*C.R. Acad. Sci., Paris*, 1948, **226**, 486–488) for the ground state of this molecule lead to similar conclusions.

† It has been pointed out by Vittum & Brown (1947) that the relative effects of a hindering substituent on the energies of the ground state and the higher energy states of a molecule should influence the direction of the wave-length shift due to steric hindrance, and shifts to lower frequencies might be expected under certain conditions. A bathochromic shift has, in fact, been observed by Brunings & Corwin (1942) and by Brooker *et al.* (1947) in certain sterically hindered pyrrole dyes. It is possible that diminished absorption *intensity* (as defined by Mulliken 1939) is a more sensitive test of steric hindrance than changes in frequency, as is suggested by Remington (1945) in work on dimethylaniline derivatives, and by the results reported in this communication. Further, it is clear that changes in the vibrational energy of a molecule on substitution may influence the maximum of an apparently smooth absorption band in a complex manner. Thus band shifts resulting from substitution require cautious interpretation.

It was found that solutions of most of the compounds examined were unstable when left in the light, and changes were observed in the absorption curve during measurement. 4-Dimethylaminostilbene, previously investigated by Hertel & Lührmann (1939), showed a considerably higher wave-length and extinction maximum immediately after preparing the solution than when examined after keeping for a few hours. Thus, repeated measurements on fresh solutions gave a band maximum at 346 mμ ($\epsilon = 30{\cdot}8 \times 10^3$), whereas Hertel & Lührmann's maximum lies at 332 mμ ($\epsilon = 27{\cdot}0 \times 10^3$). Our earlier results, obtained without special precautions, varied over a considerable range of wave-length and extinction. Our final result for 4′-dimethylamino-1:4-diphenylbutadiene ($\lambda_{max.} = 367$ mμ; $\epsilon = 42{\cdot}3 \times 10^3$) also lies at slightly longer wave-lengths and higher extinction than that found by Hertel & Lührmann ($\lambda_{max.} = 362$ mμ; $\epsilon = 30{\cdot}7 \times 10^3$).

TABLE 13

compound	absorption maximum (long wave-length band) wave-length (mμ)	extinction $\epsilon \times 10^{-3}$
4-dimethylaminostilbene	346	30·8
4-dimethylamino-2′-methylstilbene	343	26·5
4-dimethylamino-2′-ethylstilbene	338	25·6
4-dimethylamino-2′-*n*-propylstilbene	333	23·6
4-dimethylamino-2′-*iso*propylstilbene	330	26·0
4-dimethylamino-2′:5′-dimethylstilbene	338	24·3
4-dimethylamino-2′:5′-di*iso*propylstilbene	345	28·2
4-dimethylamino-2′:6′-dimethylstilbene	309	25·1
4-dimethylamino-2′:4′:6′-trimethylstilbene	310	27·0
4-dimethylamino-2:2′-dimethylstilbene	338	21·3
4-dimethylamino-4′-methylstilbene	345	31·2
4′-chloro-4-dimethylaminostilbene	355	31·4
2′-chloro-4-dimethylaminostilbene	356	30·3
4-dimethylamino-α:β-diethylstilbene	263	17·6
2:4:6-trimethylstilbene	282	18·9
2:4:6:2′:4′:6′-hexamethylstilbene	264	8·3
4′-dimethylamino-1:4-diphenylbutadiene	367	42·3

In order to test the effect of light on one of the compounds, the 2′:5′-dimethyl derivative was irradiated in ethyl alcohol, in *cyclo*hexane and in benzene solution with the full light of a high-pressure mercury arc (*a*) in the presence of oxygen, and (*b*) with the exclusion of oxygen, for periods of from 90 min. to 31 hr. In all experiments, with increase in irradiation the absorption band moved rapidly to shorter wave-lengths and lower extinctions ($\epsilon_{max.} = 17{\cdot}6 \times 10^3$; $\lambda = 310$ mμ after 90 min. irradiation in alcohol). Comparison with the known effect of ultra-violet light on *cis* and on *trans* stilbene (Smakula, 1934; Lewis, Magel & Lipkin 1940) suggests that the most probable explanation of this result is a *trans* to *cis* change, with the production of an equilibrium mixture of the *cis* and *trans* isomers. Solutions must therefore be freshly prepared and protected from light.

Examples of the results obtained for this series of compounds are shown in figures 1 to 5, and the maximum wave-length and extinction for the long wave-length band in each compound are given in table 13. Since the band maxima are broad, they are difficult to determine with an accuracy greater than ± 15A and are expressed in mμ. The molecular extinction coefficient ϵ is used throughout, i.e. concentrations expressed in gram-molecules per litre; 1 cm. cell.

10. *Discussion*

The results obtained in this preliminary series suggest that a number of factors may complicate the interpretation of these absorption spectra. The asymmetry of the long wave-length absorption band for 4-dimethylaminostilbene (figure 1), and changes in the shape of this band on substitution, lead one to suspect that changes in vibrational structure are influencing the outline of the band envelope to a considerable extent. Thus, for a number of the spectra shown in figures 1 to 5, the difference between the slopes of the curves is

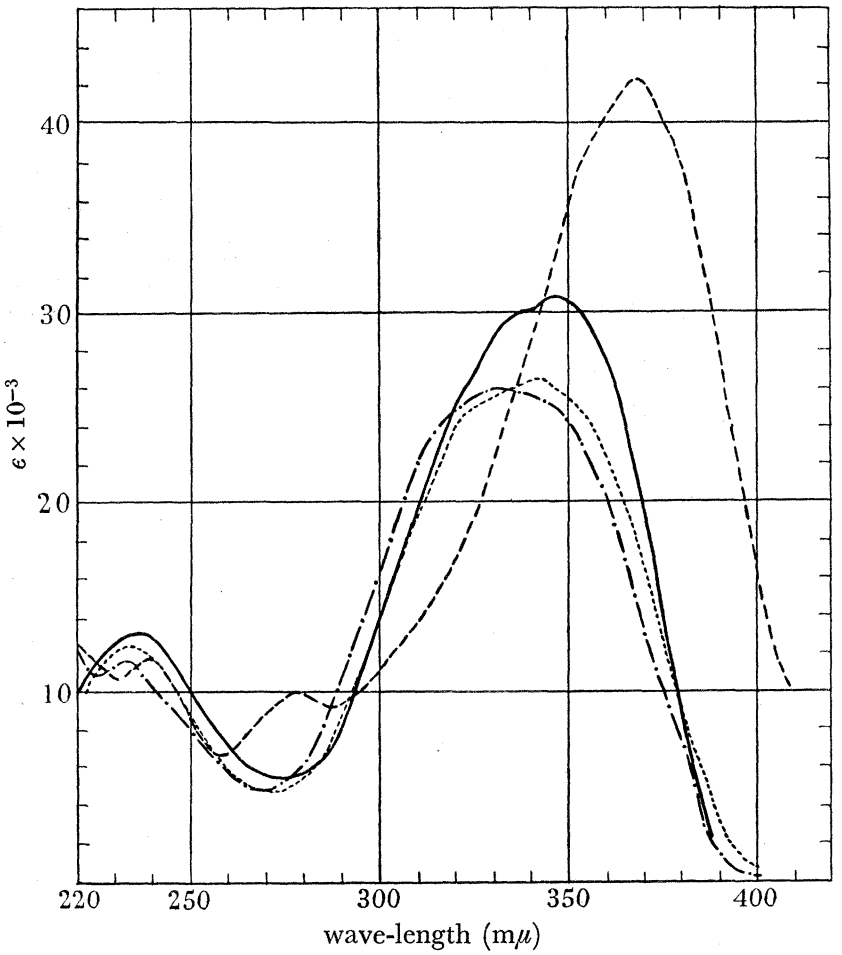


FIGURE 1. —— 4-dimethylaminostilbene.
···· 4-dimethylamino-2′-methylstilbene.
–·–· 4-dimethylamino-2′-*iso*propylstilbene.
– – – 4-dimethylamino-1:4-diphenylbutadiene.

less than the difference in $\lambda_{max.}$ given in table 13, and this must be remembered when comparing the data. The effect is more accurately represented when frequencies (or wave-numbers, $1/\lambda$) are used instead of wave-lengths in plotting the graphs. Infra-red investigations now in progress, and examination of the ultra-violet absorption at low temperatures and in different solvents, should throw light on these changes. *trans* Stilbene itself shows some rather ill-defined structure in its long wave-length band, with maxima at 319, 306 and 294 mμ in benzene at room temperature (Hausser, Kuhn & Smakula 1935) and at approximately 308, 294 and 280 mμ in *iso*octane at $-90°$ C. (Lewis *et al.* 1940).

It is also probable that the influence of substitution on the absorption spectrum may be different in the two benzene rings and at various positions in each ring. In spite of these complications, however, the effect of steric hindrance is evident in the spectra of a number of the compounds examined.

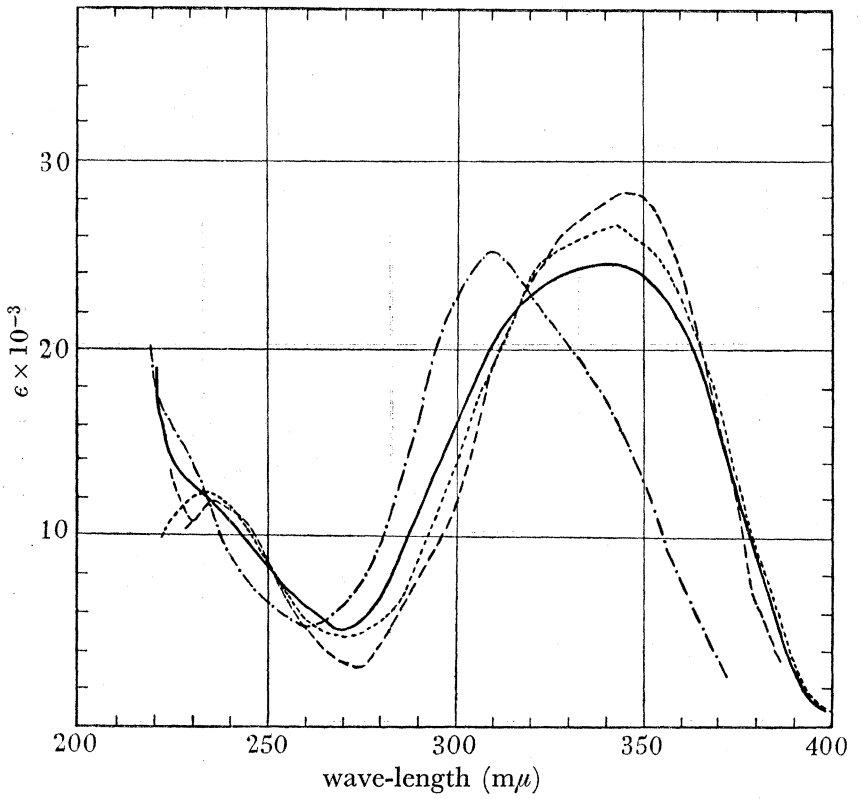


FIGURE 2. · · · · 4-dimethylamino-2′-methylstilbene.
—— 4-dimethylamino-2′:5′-dimethylstilbene.
– – – 4-dimethylamino-2′:5′-di*iso*propylstilbene.
– · – · 4-dimethylamino-2′:6′-dimethylstilbene.

Figure 1 shows the spectra of 4-dimethylaminostilbene and of its 2′-methyl and 2′-*iso*-propyl derivatives. The difference between the long wave-length bands of the 2′-methyl-derivative and the parent compound is probably negligible as far as wave-length shifts are concerned, although there is a reduction in maximum extinction on substitution. A methyl group substituted at the 4′-position produces no important changes in extinction or wave-length (figure 5). The spectrum of the 2′-*iso*propyl derivative is shifted to shorter wave-lengths however (a hypsochromic shift), the difference being greater for the peak of the curve (16 mμ) than for its slopes (about 7 mμ).

The spectra of 2′:5′-dimethyl- and 2′:5′-di*iso*propyl-4-dimethylaminostilbene are given in figure 2. There is a small hypsochromic shift due to the second methyl substituent, and a reduction in extinction maximum which also affects the shape of the curve. Preliminary examinations of the 2′:3′- and 3′:4′-dimethyl derivatives suggest that a methyl group substituted at the 3′- (or 5′-) position normally produces this small hypsochromic effect. In the 2′:5′di*iso*propyl derivative, however, the maximum is shifted 15 mμ towards longer

wave-lengths compared with the 2′-*iso*propyl compound (a bathochromic shift) and the maximum extinction is increased, so that the spectrum is similar to that of 4-dimethylaminostilbene.

The usual result of substitution of a saturated alkyl group in an aromatic molecule is a small bathochromic shift of the spectrum, provided that the group causes no steric interference with conjugation. It might be concluded from our results that the large *iso*propyl group can exert its expected bathochromic effect unhindered when substituted at the 5′-position, but that on substitution at the 2′-position this wave-length shift is reduced owing to the hypsochromic effect of steric interference with the β-hydrogen atom. This

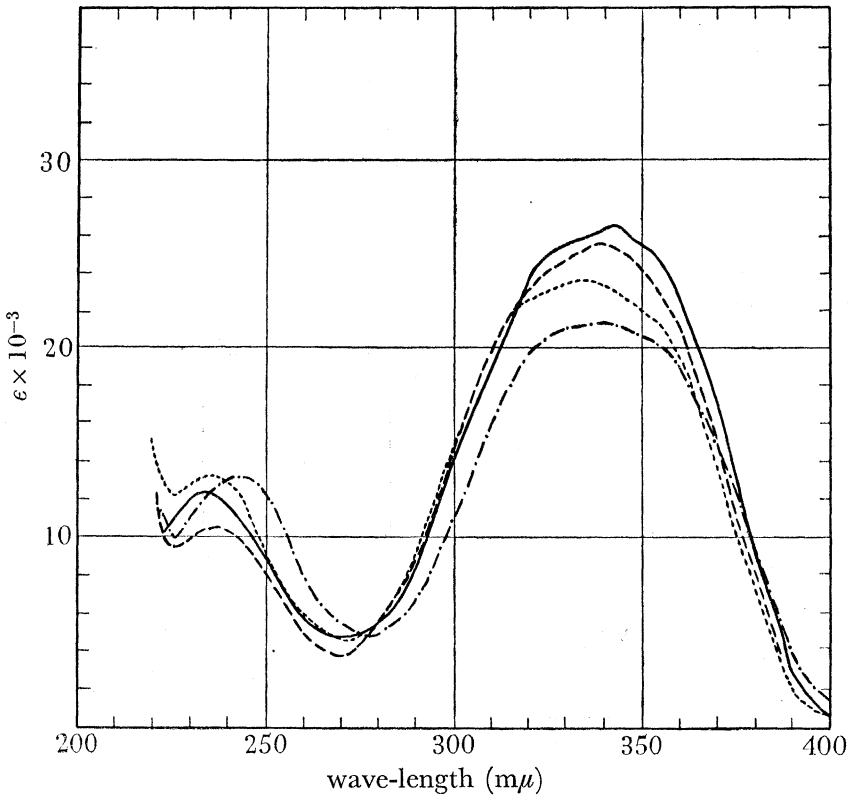


FIGURE 3. —— 4-dimethylamino-2′-methylstilbene.
– – – 4-dimethylamino-2′-ethylstilbene.
· · · · 4-dimethylamino-2′-*n*-propylstilbene.
–·–· 4-dimethylamino-2:2′-dimethylstilbene.

could account for the resultant hypsochromic shift in the 2′-*iso*propyl derivative and the smaller shifts in the same direction shown by the 2′-*n*-propyl and 2′-ethyl compounds (figure 3). This conclusion is rendered uncertain, however, by evidence from the dimethyl derivatives substituted at the 5′-position, where the small hypsochromic shifts cannot be due to steric hindrance. Further, in 4-dimethylamino-2:2′-dimethylstilbene, the only compound examined so far with a substituent in the same ring as the dimethylamino group, there is a small hypsochromic shift of the band maximum, and a bathochromic shift of the slopes of the band which may be connected with its considerably reduced extinction and the greater symmetry (figure 3). In view of this varying 'position effect' of methyl substitution in these spectra, it seems unwise to regard the fairly small spectral shifts caused

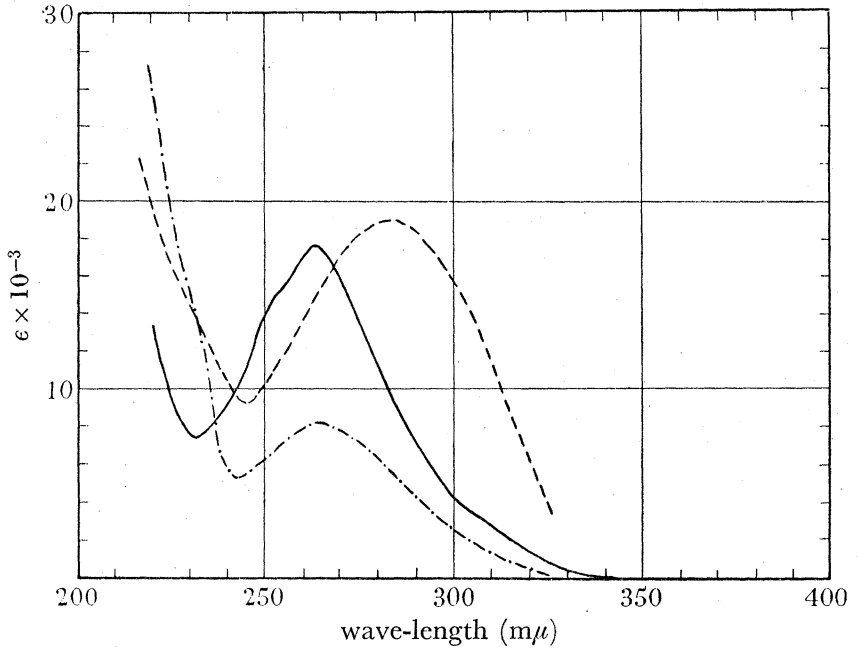


FIGURE 4. – – – 2:4:6-trimethylstilbene.
–·–· 2:4:6:2′:4′:6′-hexamethylstilbene.
—— 4-dimethylamino-α:β-diethylstilbene.

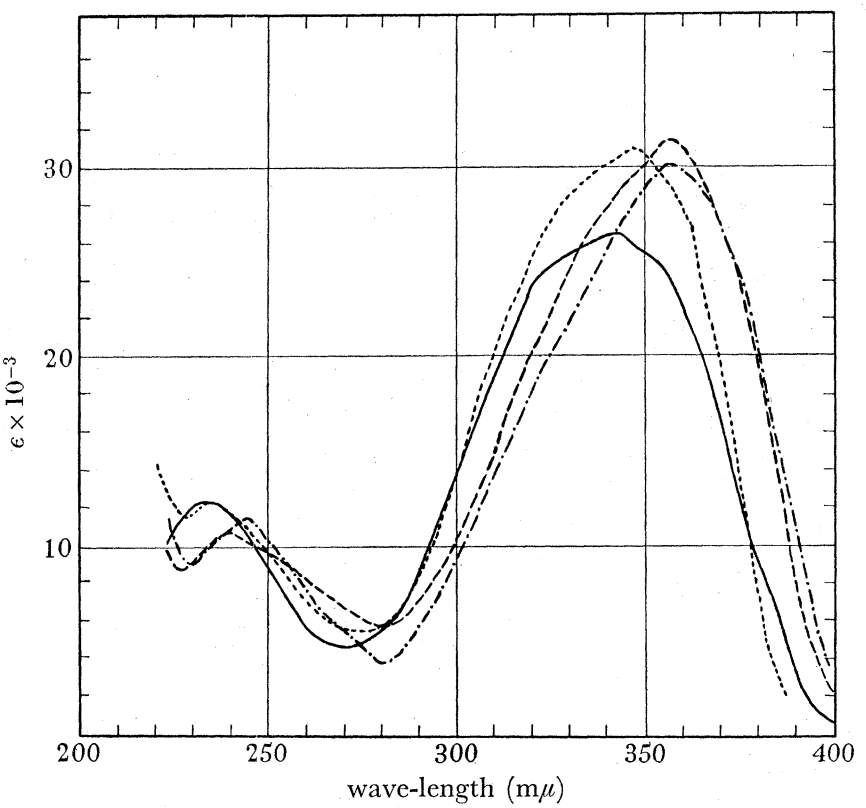
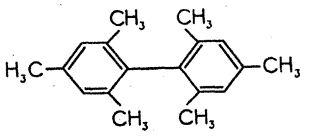


FIGURE 5. —— 4-dimethylamino-2′-methylstilbene.
···· 4-dimethylamino-4′-methylstilbene.
–·–· 2′-chloro-4-dimethylaminostilbene.
– – – 4′-chloro-4-dimethylaminostilbene.

by bulky alkyl substituents at the 2′-position as conclusive evidence of steric hindrance. Additional evidence will be obtained from the spectra of other alkyl derivatives.

In the spectrum of 4-dimethylamino-2′:6′-dimethylstilbene (figure 2) the hypsochromic shift resulting from substitution of a second methyl group is much more pronounced. The band maximum lies at 309 mμ, a shift of 37 mμ compared with 4-dimethylaminostilbene, and it is concluded that the second (6′) *ortho* methyl substituent causes considerable steric interference with the α-hydrogen atom, with consequent reduction in the planarity of the molecule. It is interesting to note that the 2′:4′:6′-trimethyl derivative gives a closely similar spectrum as far as shape and wave-length of the band maximum is concerned, but with increased extinction (table 13), a result comparable with the difference between the 2′- and 4′-methyl compounds (figure 5). It is clear that a methyl group substituted at the 4′-position will have no effect on steric conditions in the molecule.

For comparison with these substances, 2:4:6-trimethylstilbene was examined (figure 4), and its spectrum also shows a fairly large hypsochromic shift (13 mμ) compared with *trans* stilbene. In 2:4:6:2′:4′:6′-hexamethylstilbene (figure 4) the spectrum is shifted into the benzene absorption region, and it is apparent that steric hindrance between the α- and β-hydrogen atoms and the *ortho* methyl groups is so great that conjugation through the ethylenic double bond is prevented; its spectrum is similar to that of dimesityl (X) recorded



X

by Pickett, Walter & France, (1936). The same effect is apparent in the spectrum of 4-dimethylamino-$\alpha\beta$-diethylstilbene (figure 4), where it is clear that ethyl substituents on the α- and β-carbon atoms interfere with *ortho* hydrogen atoms in the benzene rings, as in $\alpha\beta$-dimethylstilbene quoted earlier (Arends 1931; Jones 1943). All these spectra are shifted into the absorption region of benzene or of its alkyl derivatives (cf. *n*-propylbenzene (Hillmer & Schorning 1934) or ethylbenzene (Jones 1943)).

The spectra of other compounds of interest for comparison with those already described are given in figures 1 and 5. 4′-Dimethylamino-1:4-diphenylbutadiene (figure 1) shows a bathochromic shift of 21 mμ and an increased extinction maximum compared with 4-dimethylaminostilbene, and this is consistent with changes observed on lengthening the conjugated chain in unsubstituted diphenylpolyenes (Hausser *et al.* 1935). We have also observed in this compound an additional band at 238 mμ not recorded by Hertel & Lührmann (1939), while our determination of their 271 mμ band places it at longer wave-lengths (277 mμ). The spectra of 2′- and 4′-chloro-4-dimethylaminostilbene are given in figure 5, together with those of the corresponding methyl derivatives, and it is seen that a 9 to 10 mμ bathochromic shift results on introducing a chlorine atom at the 2′- or 4′-positions.

The results discussed in this section indicated that an examination of molecular models and plane diagrams of these molecules would be of interest, although evidence from these could not be regarded as conclusive. A number of uncertainties are involved in their construction, e.g. space accommodation of atoms through interpenetration of electron clouds is difficult to predict and atomic constants evaluated for molecules in the ground state

would be expected to be modified in the excited states, so that one might assume less effective steric hindrance in the real molecule than in the model. On the other hand, when a dimethylamino group is substituted at the 4-position in stilbene, increased double-bond character is expected in the bond between an ethylenic carbon atom and the neighbouring ring carbon, and steric interference should be increased on this account. Further, Jones (1945) and Wepster (1946) have pointed out that considerable inaccuracy is involved in the construction of some model atoms of the Stuart type, owing to Stuart's alteration of the van der Waals radii of these atoms to enable them to 'fit together' (Stuart 1934).

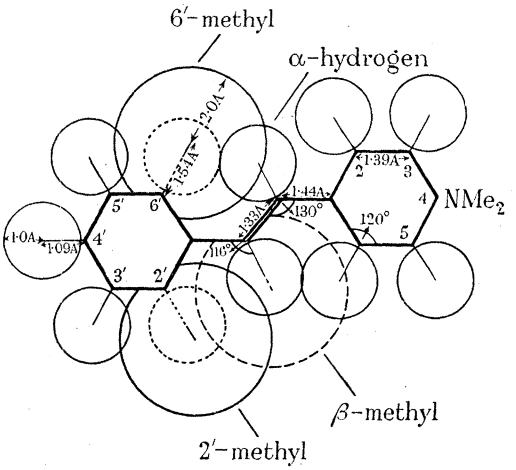


FIGURE 6. Generalized diagram of 4-dimethylamino-β: 2′: 6′-trimethylstilbene.

The plane diagram in figure 6 is based on dimensions for the stilbene molecule taken from the X-ray crystallographic data of Robertson & Woodward (1937). It is seen that a methyl group substituted at the 2′-position appears to interfere slightly with the β-hydrogen atom, although comparison of the $\lambda_{max.}$ of the 2′- and 4′-methyl derivatives and the parent compound suggests that this interference is reduced by mutual accommodation of the atoms in the real molecule.* When the substituents are reversed, as in α- (or β-) methylstilbene, the single methyl group interferes with hydrogen atoms at both the 2- and 2′-positions in the rings, with the resulting spectral shift quoted earlier (Arends 1931; Jones 1943). A similar degree of interference is produced by substitution of two methyl groups at both 2′- and 6′-positions, or by three groups at the 2′-, 4′- and 6′-positions, since a 4′ substituent has no effect on steric conditions in this molecule. The diagram shows a further increase in steric hindrance in $\alpha\beta$-dimethylstilbene (and its 4-dimethylamino derivative) with substituents on both α- and β-carbon atoms, and in 2:4:6:2′:4′:6′-hexamethylstilbene in which the four *ortho* positions are substituted.

Using Fisher-Hirschfelder-Taylor models of the Stuart type, it appears to be possible to construct a planar molecule with a methyl substituent on the α-carbon atom, but there is considerable hindrance to the free rotation of the methyl group and of neighbouring parts

* The $\epsilon_{max.}$ (and absolute intensity) in the 2′-methyl compound is diminished, however. If intensity changes are a more sensitive test of steric interference than changes in $\lambda_{max.}$, this observation would suggest that the small steric effect indicated by the plane diagram is present in the real molecule in solution.

of the molecule. When the α-substituent is larger than a methyl group, or when both α- and β-hydrogen atoms are substituted, a planar molecule seems an impossibility as is shown in the 4-dimethylamino-αβ-diethylstilbene model (figure 7B). There is little steric hindrance in models of the 2′-methyl and 2:2′-dimethyl compounds, and none in the 2′-chloro derivative. A planar model of the 2′:6′-dimethyl derivative can be constructed but appears less probable than in the case of the 2:2′-dimethyl compound, for it is easily buckled by rotation of the more hindered methyl group, i.e. 6′-methyl in figure 6. In figure 7 are shown the 4-dimethylaminostilbene model (A), and planar (C) and non-planar (D) models of the 2′:6′-dimethyl derivative. The buckling in (D) has been caused by rotation of the 6′-methyl group, thus forcing the α- and β-hydrogen atoms out of the plane of the benzene rings. This steric interference will presumably become greater when the two further *ortho* hydrogen atoms are substituted by methyl groups. Planar models of the 2′-alkyl derivatives can also be constructed, although there is clearly hindrance to the free rotation of the alkyl group when it is larger than methyl.

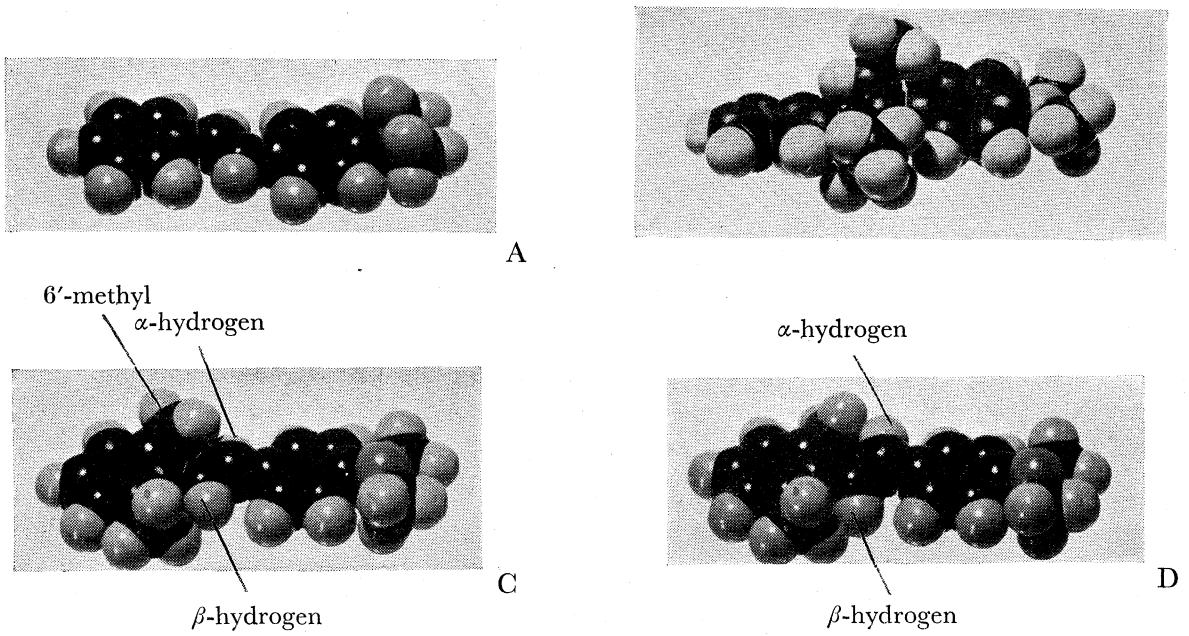


FIGURE 7. Fisher-Hirschfelder-Taylor molecular models to show: A, the planar character of 4-dimethylaminostilbene; B, the lack of planarity in 4-dimethylamino-αβ-diethylstilbene; C and D, possible planar and non-planar forms respectively of 4-dimethylamino-2′:6′-dimethylstilbene.

Thus an examination of molecular models for this series of compounds indicates a varying degree of steric interference between groups in the region of the ethylenic double bond, which results in restriction of the rotation of substituents in many compounds and a buckling of the molecule for other derivatives. However, conclusions derived from spectroscopic data suggest that steric hindrance effects observed in the molecular models are less than those operating in the actual molecules, probably on account of limitations involved in the construction of the models. Although a planar model can be made, for example, for hexamethylstilbene, the spectrum shows definite lack of conjugation between the benzene rings, from which it is concluded that the molecule is non-planar. Evidence

from diagrams, models and spectra suggests that steric interference with the planar configuration of molecules in this series varies continuously from the planar 4-dimethylaminostilbene molecule and its chloro-derivatives to the seriously buckled $\alpha\beta$-diethyl derivative.

When the evidence concerning steric conditions in the molecules is compared with the biological activities of the compounds discussed in this section a close connexion is suggested between lack of inhibitory power and buckling of the molecule. In the 4-dimethylaminostilbene derivatives with substituents on the α- and β-carbon atoms of the ethylenic double bond, or with methyl groups at two *ortho* positions in a phenyl group, steric factors reduce the planarity of the molecule, thus affecting the conjugation resonance absorption characteristic of the whole molecule. These compounds are found to be non-inhibitory. Bulky alkyl substituents at the 2′-position may influence the molecule in a similar manner, but more evidence is required on this point. It is certainly of interest that 2′-*n*-propyl- and 2′-*iso*propyl-4-dimethylaminostilbenes are inactive compounds and that the 2′-ethyl derivative is only weakly active.

IV. The carcinogenic action of 4-aminostilbene and derivatives

The inhibitory action of 4-aminostilbene and related compounds, although more intense than that shown by carcinogens of the class of condensed polynuclear aromatic hydrocarbons (cf. Badger *et al.* 1942), was judged to be essentially similar to the latter in the qualitative sense. Since no aminostilbenes had previously been examined for carcinogenicity, it was felt that the present work, if extended to include such an examination, would provide a unique opportunity of testing the suggested etiological association between these two properties. The results of these experiments are summarized in tables 14 and 15. The first included 4- and 4′-dimethylaminobenzylideneaniline (neither of which appeared to have any inhibitory activity), and the inhibitory compounds 4-amino-, 4-acetamido-, 4-dimethylamino- and 4-diethylaminostilbene. These substances were administered to albino rats by subcutaneous injection, in the flank, of solutions in arachis oil (see table 14 for details), in every case save one in which 4-dimethylaminostilbene was tested by incorporation in the diet (experiment 124*b*). Using the first two compounds named, no tumours had been recorded when the experiments were terminated (perhaps a little prematurely) at the 409th and 465th days respectively; in all other cases such experiments were allowed to continue to their natural conclusion. With all the inhibitory compounds tested, a few sarcomata appeared at or near the site of injection, as well as remote tumours mainly comprising carcinomata of the external acoustic duct, mammary adenomata, and cholangiomata. That these tumours were in fact induced is made certain by their number, nature and distribution, although their latent period was in all cases relatively long (from 7 to 18 months); sarcoma and cholangioma are unknown in this stock, and mammary adenoma excessively rare (only one spontaneous case has been observed in several tens of thousands of animals examined). Even more striking, squamous keratinizing carcinoma of the external acoustic duct does not appear to occur in the rat in any circumstances *de novo*, and has previously been described only as a result of administration of the carcinogens 2-anthramine and 2-acetamidofluorene (Bielschowsky 1947). Bearing in mind its natural

TABLE 14

experiment number	compound	initial number of rats	dosage and route of administration	tumours recorded: at or near injection site	tumours recorded: distant	approx. latent period (days)	remarks
120	4-dimethylamino-benzylideneaniline	12	5 × 5 mg. solution in arachis oil subcutaneously at approx. 2-week intervals	0	0	—	4 surviving at day 409, when experiment terminated
121	4′-dimethylamino-benzylideneaniline	12	11 × 5 mg. solution in arachis oil subcutaneously at approx. 1-week intervals	0	0	—	2 surviving at day 465, when experiment terminated
122	4-aminostilbene	12	8 × 5 mg. solution in arachis oil subcutaneously at approx. 1-week intervals	sarcoma	0	372	—
				sarcoma	0	440	—
				0	2 mammary adenomata	447	figure 8, plate 2
				0	2 mammary adenomata	449	—
123	4-acetamido-stilbene	12	3 × 2 mg. solution in arachis oil subcutaneously at approx. 1-month intervals	sarcoma	0	339	transplanted
				sarcoma	0	339	experiment terminated at day 555
				0	carcinoma acoustic duct	436	—
124*a*	4-dimethylamino-stilbene	12	8 × 5 mg. solution in arachis oil subcutaneously at approx. 1-week intervals	sarcoma	0	212	—
				0	lung adenoma cholangioma	216	—
				sarcoma	carcinoma acoustic duct	508	—
124*b*	4-dimethylamino-stilbene	12	0·5 mg./rat/day in diet for 2 months, then 0·25 mg./rat/day in diet for 4 months	0	carcinoma acoustic duct (right ear)	267	—
				0	carcinoma acoustic duct (left ear)	270	—
				0	carcinoma acoustic duct (right ear) lung adenoma	277	—
				0	carcinoma acoustic duct (right ear)	284	—
				0	carcinoma acoustic duct (right ear)	329	—
				0	cholangioma	observed day 330	
				0	2 carcinomata acoustic duct (bilateral)	370	—
				0	squamous carcinoma of jaw cholangioma	371	—
				0	2 carcinomata acoustic duct (bilateral) cholangioma	386	experiment terminated at day 424
				0	carcinoma acoustic duct (right ear) mammary adenoma cholangioma lung adenoma	observed day 424	—
125	4-diethylamino-stilbene	12	8 × 5 mg. solution in arachis oil subcutaneously at approx. 1-week intervals	sarcoma	carcinoma acoustic duct	killed day 448	experiment terminated at day 538
				0	lung adenoma	killed day 476	—
				sarcoma	carcinoma acoustic duct cholangioma	killed day 538	figures 9 to 11, plate 2

rarity or even non-occurrence spontaneously, the fact that this remarkable tumour is so frequent and characteristic a result of treatment with aminostilbenes as well (e.g. being especially prominent in experiment 124*b* in which 4-dimethylaminostilbene was fed in the diet), strongly suggests some common feature in the carcinogenic action of aminostilbenes and aminofluorenes; this point will be further considered below. This first series of experiments therefore revealed the carcinogenic action of various aminostilbenes in the albino rat; of seventy-two animals exposed to the action of one or other of four such compounds, twenty-three developed a total of eight sarcomata and thirty distant tumours as a result.

In the second series of experiments (table 15), 4-amino-, 4-dimethylamino- and 4-dimethylamino-2′-methylstilbenes, with 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)-ethylene, were examined by repeated subcutaneous injection of solutions in arachis oil in male and female mice and rats. These experiments bring out the contrast between the carcinogenicity of these compounds in the two rodent species: of 120 rats exposed, forty-eight developed a total of twenty sarcomata and fifty-one tumours (again mainly carcinomata of the acoustic duct, cholangiomata and mammary adenomata), in organs distant from the site of injection, while of 120 mice similarly treated, only four sarcomata and two distant tumours were recorded in five. While no conclusion is possible, it is tempting to speculate that this divergence may reflect the similar difference already noted in the reaction of the two species to the inhibitory property of the aminostilbenes, and which may conceivably depend upon some metabolic difference, the nature of which is still, however, unknown. From table 15 it would also seem there is no obvious sex difference in the induction of sarcomata with these compounds in the rat, but that the development of mammary adenomata is, as one might anticipate, largely confined to the female sex. Further, the incidence of mammary adenomata was almost certainly correlated with damage to the liver, although not necessarily with the presence of cholangiectasis. In other cases (cf. Cantarow, Paschkis, Stasney & Rothenberg 1946), this phenomenon has been related with an increase in endogenous oestrogen due to interference with the function of the liver in the intermediary metabolism and excretion of the sex steroids. Lastly, the compounds themselves proved roughly similar in the nature and extent of their carcinogenicity, and in the relatively long latent period of the tumours induced. An exception does, however, seem to be provided by 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)-ethylene, which produced a greater yield of sarcomata, and a considerably lower incidence of remote tumours, with latent periods distinctly less than a year in most instances, rather than more than a year as in the case of the other compounds examined.

V. General discussion

From earlier papers, there seemed little doubt of a substantial correlation between carcinogenicity and growth-inhibitory power in the polycyclic aromatic hydrocarbons, and it therefore appeared a possibility, as in the case of carcinogenic agents such as X-rays and radium, that the mode of action of chemical carcinogens might be indirect, and that they could operate by retardation of the growth of normal cells, the latter eventually reacting to give a new cell race with an increased rate of fission. While the correlation on which this

TABLE 15

experiment number	compound	initial number of animals	dosage and route of administration	tumours recorded: at or near injection site	tumours recorded: distant	approx. latent period (days)	remarks
126	4-aminostilbene	12♂ albino rats	7 × 2 mg. solution in arachis oil subcutaneously at approx. 1-week interval	0	mammary adenoma	423	—
				sarcoma	0	436	
		12♀ albino rats	7 × 2 mg. solution in arachis oil subcutaneously at approx. 1-week intervals	0	carcinoma acoustic duct	163	—
				sarcoma	carcinoma acoustic duct	observed day 343	—
				0	mammary adenoma	—	—
				sarcoma	0	391	—
				0	carcinoma acoustic duct	415	—
				0	mammary adenoma	425	—
				sarcoma	cholangioma	428	—
				0	mammary adenoma	430	—
				sarcoma	mammary adenoma	488	—
127	4-aminostilbene	12♂ stock mice	9 × 0·5 mg. solution in arachis oil subcutaneously at 1- or 2-week intervals	0	0	—	of 24 mice, 22, 19 and 8 survived at 6, 12 and 18 months respectively from the beginning of the experiment
		12♀ stock mice	9 × 0·5 mg. solution in arachis oil subcutaneously at 1- or 2-week intervals	0	0	—	—
128	4-dimethylaminostilbene	24♂ albino rats	6 × 5 mg. solution in arachis oil subcutaneously at 2- to 4-week intervals	0	cholangioma	214	figures 12 to 16, plates 3 and 4
				0	carcinoma acoustic duct	266	—
				fibrosarcoma	0	382	—
				sarcoma	intrathoracic carcinoma (? bronchogenic)	sarcoma observed at day 431	in a description of tumours induced by 2-acetamidofluorene in the rat, Harris (1947) mentions a similar epidermoid carcinoma of the lung
				0	carcinoma acoustic duct	432	—
				lymphosarcoma	0	446	—
				0	carcinoma acoustic duct	502	—
		24♀ albino rats	6 × 5 mg. solution in arachis oil subcutaneously at 2- to 4-week intervals	0	cholangioma	detected day 357	—
				0	cholangioma	detected day 375	—
				sarcoma	cholangioma	sarcoma observed day 401	—
				0	mammary adenoma	—	—
				0	mammary adenoma	436	transplanted
				0	carcinoma acoustic duct	479	—
				0	carcinoma acoustic duct	480	—
				0	cholangioma	481	—
				0	3 mammary adenomata	482	—
				0	6 mammary adenomata	483	—
				0	7 mammary adenomata	484	—

129	4-dimethylaminostilbene	24♂ stock mice	11 × 1 mg. solution in arachis oil subcutaneously at intervals of 1 to 3 weeks	sarcoma	0	420	21, 16 and 9 mice survived at 6, 12 and 20 months respectively from the beginning of the experiment
		24♀ stock mice	11 × 1 mg. solution in arachis oil subcutaneously at intervals of 1 to 3 weeks	sarcoma	0	270	21, 13 and 8 mice survived at 6, 12 and 20 months respectively from the beginning of the experiment
130	4-dimethylamino-2′-methylstilbene	12♂ albino rats	4 × 5 mg. solution in arachis oil subcutaneously at intervals of several weeks	sarcoma	0	270	—
				0	cholangioma	345	—
				0	ganglioneuroma of adrenal	observed at day 404	—
				sarcoma	0	418	—
				0	carcinoma acoustic duct	420	—
		12♀ albino rats	4 × 5 mg. solution in arachis oil subcutaneously at intervals of several weeks	0	mammary adenoma	288	—
				0	sarcoma of liver	320	associated with taeniasis
				0	cholangioma	405	—
				0	carcinoma acoustic duct	425	—
				0	mammary adenoma	—	—
				0	cholangioma	462	—
				0	mammary adenoma	463	—
				0	cholangioma	—	—
131	4-dimethylamino-2′-methylstilbene	12♂ stock mice	8 × 1·25 mg. solution in arachis oil subcutaneously at intervals of 2 to 3 weeks	0	0	—	9, 5 and 2 mice survived at 6, 12 and 17 months respectively from the beginning of the experiment
		12♀ stock mice	8 × 1·25 mg. solution in arachis oil subcutaneously at intervals of 2 to 3 weeks	0	0	—	9, 9 and 7 mice survived at 6, 12 and 17 months respectively from the beginning of the experiment
132	1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene	12♂ albino rats	6 × 5 mg. solution in arachis oil subcutaneously at intervals of 2 to 3 weeks	sarcoma	0	215	—
				sarcoma	0	261	transplanted
				sarcoma	0	263	transplanted
				sarcoma	0	289	—
				0	hypernephroma	383	figures 17 to 19, plate 4
		12♀ albino rats	6 × 5 mg. solution in arachis oil subcutaneously at intervals of 2 to 3 weeks	sarcoma	0	252	—
				sarcoma	0	253	transplanted
				sarcoma	0	256	—
				sarcoma	0	366	—
				sarcoma	mammary adenoma	369	—
				sarcoma	0	406	also polycystic kidneys
133	1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene	12♂ stock mice	8 × 1·0 mg. solution in arachis oil subcutaneously at intervals of 2 to 3 weeks	sarcoma	0	109	11, 6 and 2 mice survived at 6, 12 and 18 months respectively from the beginning of the experiment
		12♀ stock mice	8 × 1·0 mg. solution in arachis oil subcutaneously at intervals of 2 to 3 weeks	0	sarcoma of uterus	136	12, 9 and 6 mice survived at 6, 12 and 18 months respectively from the beginning of the experiment
				0	lung adenoma	—	—

view is based seems sufficiently strong, undoubted exceptions occur which, if they are not enough to invalidate the hypothesis as a whole, indicate that we should regard it as a general approximation. Additional support for the general thesis has, however, been provided by the discovery first of all that various derivatives of 4-aminostilbene possess such inhibitory properties *par excellence*, and only later that these compounds are in fact endowed with carcinogenic properties; if no proof can be claimed, the correlation would seem to be more than one of chance alone.

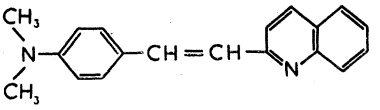
Another biological association which receives considerable support from the present results is that between carcinogenicity and the property of inducing the formation of haemolymph nodes in the rat. The spontaneous occurrence of haemolymph nodes in this species had been described by earlier workers (e.g. Vincent & Harrison 1897; Drummond 1900; Lewis 1903; Macmillan 1928), attention being drawn especially to two such nodes in the vicinity of the kidneys. Following observations on rats treated with carcinogenic tar (Lasnitzki 1938), Lasnitzki & Woodhouse (1942) described the transformation of normal lymph nodes into haemolymph nodes in the rat by treatment with 1:2:5:6-dibenzanthracene. The appearance is that of a red discoloration commencing on the surface of the nodes, varying in extent and degree, which is to be found in all regions of the body but is particularly obvious in the mesentery. Histological examination shows a widening of the lymph spaces, the presence within them of concentrations of red blood corpuscles, and the appearance of increased numbers of macrophages, loaded with red cells and haemosiderin. Lasnitzki & Woodhouse also found haemolymph node formation as a result of treatment with either of the carcinogens 3:4-benzpyrene and 20-methylcholanthrene, and not with the non-carcinogenic anthracene or phenanthrene, and they suggested that the ability of a polycyclic hydrocarbon to produce haemolymph nodes in the rat might in some way be connected with the carcinogenic property. In a later paper (1944), the same authors found little evidence to show that new corpuscles are formed in the nodes, or that the corpuscles enter directly from the blood stream, and they assumed that the effect was rather due first to a slight but continuous filtration of red blood corpuscles from the capillaries into the surrounding tissues, and secondly, to these extravascular corpuscles being received by the peripheral lymphatics and carried along them to the regional nodes.

This curious phenomenon was observed at a very early stage in the examination of the aminostilbenes, and although its real significance is not at all apparent, it provided an additional reason, quite apart from the inhibitory property, for investigating the carcinogenic potency of such compounds. Once more, the coincident appearance of these three biological features, in an entirely new chemical class, represents an association which is most unlikely to be fortuitous.

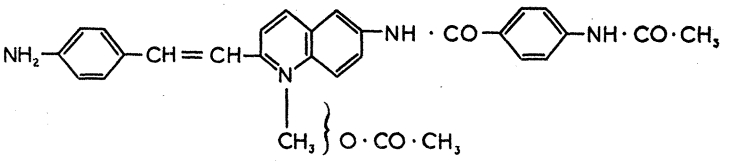
So far as concerns the tumours themselves induced by aminostilbenes, one other observation may be noted here, if only to place it on record since its underlying interpretation is once again obscure. In a small proportion of primary induced sarcomata appearing at the site of injection, regular growth was followed by a phase of regression. This behaviour appeared quite unrelated to any accident of infection, and although it had not been previously observed in the case of sarcoma induction in rats and mice by the carcinogenic hydrocarbons, is possibly akin to the spontaneous regression of histologically malignant rabbit tumours induced with 9:10-dimethyl-1:2-benzanthracene, which was described by

Beck (1945). In none of the present examples was regression ever complete, recurrence was invariable, and the histological malignancy of the tumour was confirmed in every case. These alterations were, however, most unusual, and although they affected only a small proportion of the total number of tumours examined, they are almost certainly worth further attention in the future.

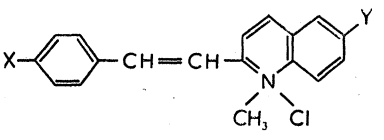
A current problem, arising from the number and variety of the known carcinogens, concerns the extent of any structural relationships between tumour-producing agents of one chemical class and those of another. The solution will clearly indicate the degree of chemical specificity of the carcinogenic process, and the extent to which any community of structure may signify a common principle of action. Even in the present investigation there have arisen a few observations which suggest, if only in the most tentative way, a certain chemical affinity between carcinogens of different classes. In the first place, attention has already been drawn to the inhibitory effects of 2-(4′-dimethylaminostyryl)-quinoline (XI). Although relatively slight, these certainly justify the examination of this compound for carcinogenicity. The experiment is now being carried out, and any positive result would have the greatest interest in relation to the known carcinogenicity of 2-(4′-aminostyryl)-6-(*p*-acetamidobenzamido)quinoline methoacetate ('styryl 430', XII), which was discovered by Browning and Gulbransen (Browning, Cohen, Cooper, Ellingworth & Gulbransen 1933) in work which arose from tests for trypanocidal activity in a series of styrylquinolinium compounds of the constitution XIII (Browning, Cohen, Ellingworth & Gulbransen 1924, 1926*a*, *b*).



XI



XII



X = NH_2 or $N(CH_3)_2$; Y = NH·CO·CH_3

or X = NH·CO·CH_3; Y = NH_2 or $N(CH_3)_2$

XIII

Since 'styryl 430' as a carcinogen has always occupied an apparently exceptional position, seemingly unconnected with carcinogens of other chemical types, the potential interest of the above relationships is sufficiently obvious.

A further suggestive circumstance is the similarity, in their nature and distribution, of the tumours now described as induced in the rat by the aminostilbenes, and those induced in the same species by 2-acetamidofluorene (Wilson, DeEds & Cox 1941; Bielschowsky 1944, 1947). The similarity applies specially to the causation by these agents of squamous

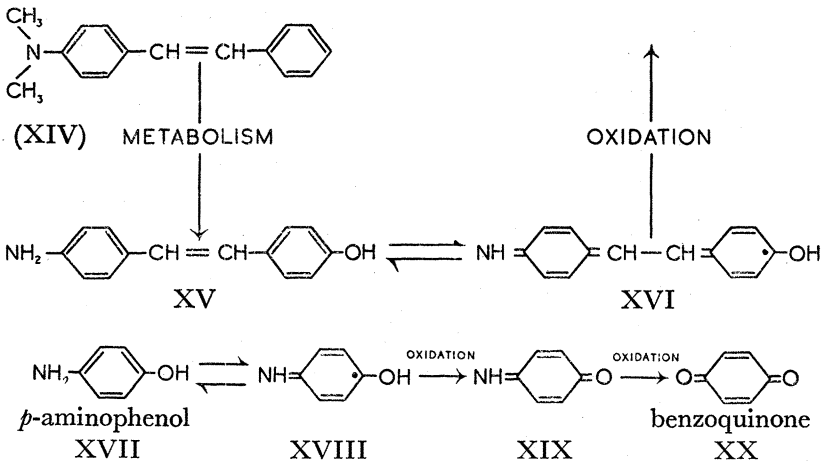
keratinizing carcinomata in various sites, of liver tumours, of tumours of the breast, of subcutaneous fibromata, and of pulmonary adenomata, although the parallelism is, of course, by no means complete. Thus while Bielschowsky finds that epithelial tissues are much more susceptible to the action of 2-acetamidofluorene than is the mesenchyme, the aminostilbenes have apparently a greater propensity than acetamidofluorene to induce sarcomata at the site of injection. Again, the tumours of the liver due to aminostilbenes are almost invariably cholangiomatous, and less malignant than the liver tumours brought about by 2-acetamidofluorene, while the mammary tumours evoked by the former substances are practically always simple, in contrast with the malignant breast tumours due to acetamidofluorene. If part of these differences may conceivably be due to strain differences in the animals used—and it has been shown both by Bielschowsky (1946) and Harris (1947) that this factor may greatly affect the type of tumours induced by acetamidofluorene—another part is no doubt due to the inherent differences of these two chemical types. Even so it is remarkable that the highly individual and characteristic squamous cell carcinoma arising in the external acoustic duct, should have been reported only in association with administration of 2-acetamidofluorene, of 2-anthramine, or of the aminostilbenes, and so far not at all as occurring spontaneously. Taken together, these facts suggest some significant relationship between 4-aminostilbene and 2-aminofluorene, perhaps similar to that between the carcinogenic 2:2′-azonaphthalene and 3:4:5:6-dibenzcarbazole (Cook, Hewett, Kennaway & Kennaway 1940).

It is interesting to compare the action of 4-dimethylaminostilbene with that of the isosteric 4-dimethylaminoazobenzene ('butter yellow', dimethyl yellow), which induces liver cancer in the rat (Kinosita 1937). Jacobi & Baumann (1942) suggested that the protection afforded by high protein and high riboflavin diets against butter-yellow carcinogenesis was partly due to the demethylation of the dye to 4-aminoazobenzene, stated by Kinosita to be non-carcinogenic. Kirby (1944, see also 1945), was, however, able to show that the methyl groups in butter yellow are not strictly essential for carcinogenesis. While preliminary evidence has been obtained (by Dr L. A. Elson) that dealkylation of 4-dimethylaminostilbene proceeds to a certain degree, it is apparent from tables 14 and 15 that there is little if any difference in the carcinogenicity of this and 4-aminostilbene, and the process therefore cannot primarily depend on the provision of labile methyl groups. To what extent the carcinogenicity of the aminostilbenes can be influenced by diet has so far only been studied in a very provisional way, and no conclusions have yet been reached.

An interesting similarity appears to exist between 4-dimethylamino-4′-methylstilbene (experiment 69) and 4-dimethylamino-4′-methylazobenzene, the corresponding and only feebly carcinogenic derivative of butter yellow, each of which has a methyl substituent in the *p*-position of ring B (see Greenstein 1947, p. 68); in both cases the blocking of this *p*-position appears to have reduced or extinguished the characteristic biological property, of inhibitory activity in the former and of carcinogenicity in the latter. As already indicated, this phenomenon in the case of the aminostilbenes is possibly due to the nature of the metabolism which compounds of this type may undergo in the body. While the metabolism of azobenzenes and aminostilbenes is no doubt radically different, and the biological fate of the latter is still largely unknown, preliminary experiments by Dr L. A. Elson have been

sufficient to indicate some of the changes which these stilbenes are likely to undergo *in vivo*; a few of these are shown in XIV to XVI for 4-dimethylaminostilbene in comparison with known steps in the oxidation of 4-aminophenol (XVII to XX). It is obvious that these are such as might readily be expected to interfere with the oxidative mechanisms of the cell.

Lastly may be mentioned a recently discovered fact, which promises to be the starting-point for fresh developments. In experiments to determine the influence of diet upon the growth-inhibitory effects of carcinogens, Elson (cf. Elson & Warren 1947) has found that this initial inhibitory action can be greatly reduced by a diet sufficiently rich in protein. In one experiment, the Walker carcinoma grew equally well in rats maintained on diets containing 5 or 25% of protein (casein); on the other hand, administration of a single dose of 2′-chloro-4-dimethylaminostilbene (150 mg./kg. intraperitoneally) produced no significant inhibition of the growth of the tumour in rats on the high-protein diet, the ratio of control and treated tumours being 1·5, while the same dose administered to rats given the 5% protein diet produced a remarkable inhibition, the corresponding ratio being no less than 34·0. Particularly since it was already known that the experimental production of cancer



of the liver by various azo compounds could be similarly delayed or prevented, by administration of a high proportion of protein in the diet, the possibility must be considered whether the initial action of chemical carcinogens may not be to inhibit growth by depleting the cellular protein, by rendering it unavailable, or possibly by interfering with its normal synthesis, that the eventual emergence of the malignant cell may be achieved through an irreversible modification of protein synthesis, and that it is this reorientation which underlies the unregulated and permanently enhanced capacity for growth. In more recent investigations of the mode of action of carcinogens there can be distinguished two complementary trends (cf. Haddow 1947), first to define the problem of carcinogenesis as essentially one of cellular nutrition, and secondly to regard all carcinogens as sources or carriers of energy in such a form as can readily interfere with the normal growth of the cell. In the advancement of such a study it is suggested that the amino-diarylethylenes may present certain advantages, not merely in their comparative simplicity and in the relative ease with which one may hope to establish a connexion between constitution and activity, but in the approach to the most fundamental aspects of the malignant transformation.

VI. Experimental: preparation of stilbenes

(with J. L. Everett and F. Goulden)

The following standard methods of preparation have been employed, but in nearly all cases the method of purification has been modified by the employment of chromatography. In particular, all the compounds submitted for biological test have been chromatographed before being crystallized (when solid) or distilled, and in some cases sublimed in a high vacuum. The melting-points given are uncorrected.

A. (1) Condensation of an aromatic aldehyde with *p*-nitrophenylacetic acid and piperidine yields a 4-nitrostilbene with or without substituents in the second ring (Pfeiffer & Sergiewskaja 1911); the nitro group can then be reduced to amino. (2) The Perkin reaction, in which potassium or sodium *p*-nitrophenylacetate is condensed with the aromatic aldehyde in presence of acetic anhydride, affords 4-nitrostilbene-α-carboxylic acids (α-phenylcinnamic acids). These are decarboxylated to stilbenes, yielding in many instances the *cis* compounds, whereas the *trans* isomerides are invariably obtained by the method A1. The nitro group can be reduced to amino by means of stannous chloride in acetic acid (Thiele & Escales 1901), and in some cases by hydrogen in presence of Raney nickel.

B. 2:4-Dinitrotoluene can be used in place of *p*-nitrophenylacetic acid and affords 2:4-dinitrostilbenes (Thiele & Escales 1901).

C. The condensation of an aryl diazonium salt with a cinnamic acid is convenient for the preparation of certain stilbenes, especially the 2- and 4-nitrostilbenes (Meerwein, Büchner & van Emster 1939; Koelsch 1943).

D. The action of benzyl magnesium chloride or one of its homologues on an aromatic aldehyde or ketone, the resulting carbinol being dehydrated, generally without purification, to the stilbene, usually by heating with acetic-hydrochloric acid (4:1, method D1) or phosphoric oxide and benzene (method D2). This method is particularly useful for the preparation of 4-dimethylaminostilbene (Sachs & Sachs 1905) and its homologues with alkyl substituents in the second ring.

E. Aminostilbenes can be converted into dimethylamino compounds by heating with methyl iodide and methyl alcohol in a sealed tube for $1\frac{1}{2}$ hr. at 100°. The quaternary salt formed is then heated with excess of 5% sodium ethoxide for 3 to 4 hr.

This investigation has been supported by generous grants to the Royal Cancer Hospital (Free) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, and the Anna Fuller Fund. Our thanks are also due to Dr C. W. Shoppee and many other colleagues for much valuable discussion and criticism; to Mr R. N. Beale, who determined the ultra-violet absorption spectra of the 2′-ethyl- and 2:2′-dimethyl-4-dimethylaminostilbenes and checked the measurements of the other spectra reported; and to Dr Harold Burton for his advice in the nomenclature of compounds newly described. The work has also been greatly assisted by Mr John Marsh, and it is a special pleasure to acknowledge his unfailing helpfulness in the preparation and conduct of the biological experiments.

TABLE 16

Solvents: A, acetic acid; B, benzene; C, *cyclo*hexane; E, ethanol; M, methanol; P, petroleum

compound	ref. no. to notes pp. 188–191	method of preparation	yield (%)	melting-point or boiling-point (°C)	solvent and appearance	found C	found H	calc. C	calc. H	formula
1:2-di-(2′:4′:6′-trimethylphenyl)ethanol		D	82	124–125	P, needles	85·3	9·5	85·1	9·3	$C_{20}H_{26}O$
2:4:6:2′:4′:6′-hexamethylstilbene		D2	good	105	E or M, stout prisms P, fine needles	90·4	9·4	90·8	9·2	$C_{20}H_{24}$
2:4:6-trimethylstilbene		D2	good	53	M, fine needles	92·0	8·3	91·9	8·1	$C_{17}H_{18}$
4-methylthiostilbene		C	12	140	C, plates	79·6	6·3	79·6	6·2	$C_{15}H_{14}S$
stilbene-4-sulphonamide		C	12	228–229	E, cubes	64·7	5·2	64·9	5·1	$C_{14}H_{13}O_2NS$
2-phenyl-1-(2′-dimethylaminophenyl)ethanol	(1)	D	66	165–167/3 mm.	—	79·4	7·5	79·7	8·0	$C_{16}H_{19}ON$
2-dimethylaminostilbene		E	good	54–55	M, plates	86·0	8·0	86·0	7·7	$C_{16}H_{17}N$
2-dimethylaminostilbene		D2	70	54–55	—	—	—	—	—	—
2-phenyl-1-(3′-dimethylaminophenyl)ethanol	(2)	D	33	171–172/3 mm.	—	79·5	8·0	79·7	8·0	$C_{16}H_{19}ON$
3-dimethylaminostilbene		D2	90	75·5–76·5	P, needles	86·1	8·0	86·0	7·7	$C_{16}H_{17}N$
3-nitrostilbene	(3)	C	37	108–108·5	E or P, yellow flattened needles	74·9	4·9	74·6	4·9	$C_{14}H_{11}O_2N$
3-aminostilbene		$SnCl_2$	71	115–116	B-P, cream prisms	—	—	—	—	—
3-dimethylaminostilbene		E	good	—	—	—	—	—	—	—
4-dimethylaminostilbene	(4)	D	—	149	B-P or E, plates	—	—	—	—	—
4-carbethoxyaminostilbene		—	good	144–145	C, felted needles	76·3	6·3	76·4	6·4	$C_{17}H_{17}O_2N$
4-benzylideneaminostilbene		—	quant.	194	E or P, yellow leaflets	89·3	6·3	89·0	6·0	$C_{21}H_{17}N$
4-phenylazostilbene		—	good	197–198	A, pale orange plates	84·8	5·7	84·5	5·7	$C_{20}H_{16}N_2$
4-(4′-dimethylaminophenylazo)stilbene		—	good	242	B, red powder	81·2	6·5	80·7	6·5	$C_{22}H_{21}N_3$
4-nitro-2′-methylstilbene		A1	47	113	E then P, yellow needles	75·3	5·6	75·3	5·5	$C_{15}H_{13}O_2N$
4-amino-2′-methylstilbene		$SnCl_2$	50	63	C, plates	85·7	7·4	86·1	7·2	$C_{15}H_{15}N$
4-acetamido-2′-methylstilbene		—	—	170–171	E, fine needles	81·4	7·0	81·6	6·7	$C_{17}H_{17}ON$
4-amino-4′-methylstilbene		$SnCl_2$	70	157–158	M, cream needles	86·1	7·3	86·1	7·2	$C_{15}H_{15}N$
4-methylaminostilbene		D1	(distilled) poor	107	ether-P, then E, buff plates	85·8	7·2	86·1	7·2	$C_{15}H_{15}N$
4-ethylaminostilbene		D1	(distilled) poor	127	M, plates	85·7	7·6	86·1	7·7	$C_{16}H_{17}N$
4-*iso*propylaminostilbene	(5)	D1	45	109–110	M, plates	85·7	8·3	86·0	8·1	$C_{17}H_{19}N$
4-diethylaminostilbene		D1	65	95–96	E, yellowish plates	85·9	8·7	86·0	8·4	$C_{18}H_{21}N$
4-diallylaminostilbene	(6)	D1	55	b.p. 240/6 mm. m.p. 68–70	M, pale yellow prisms	86·9	7·6	86·9	7·7	$C_{20}H_{21}N$
4-(ethyl-2′-acetoxyethylamino)stilbene	(7)	D1	15	56·5–57·5	M, small prisms	77·6	7·7	77·6	7·5	$C_{20}H_{23}O_2N$
4-(ethyl-2′-hydroxyethylamino)stilbene		NaOH on above	—	89·5–90·5	dil. A, plates	81·0	8·0	80·9	7·9	$C_{18}H_{21}ON$
4-dimethylamino-2′-methylstilbene		D1	71, or in poorer yield from *o*-xylyl bromide	84–85	M or P, plates	85·8	8·2	86·0	8·1	$C_{17}H_{19}N$
4-diethylamino-2′-methylstilbene		D1	70	67–68	M, yellowish rhombic plates	85·7	8·6	86·0	8·8	$C_{19}H_{23}N$
4-dimethylamino-3′-methylstilbene		D1 from *m*-xylyl bromide	20	200–215/1·5 mm. 119·5–120	B-E, plates	85·9	7·8	86·0	8·1	$C_{17}H_{19}N$
4-dimethylamino-4′-methylstilbene		D1 from *p*-xylyl bromide	15	162–164	C or M, yellowish plates or flattened needles	85·9	8·0	86·0	8·1	$C_{17}H_{19}N$
4-dimethylamino-2′:3′-dimethylstilbene		D1	60	119–120	E, flattened needles	86·0	8·5	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-2′:4′-dimethylstilbene		D1	18	126–127	B-M, yellowish plates	86·1	8·4	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-2′:5′-dimethylstilbene		D1	55	131–132	E	85·9	8·7	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-2′:6′-dimethylstilbene	(8)	D1	poor	71	M, needles	85·7	8·5	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-3′:4′-dimethylstilbene		D1	20	167	B-E	86·2	8·5	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-3′:5′-dimethylstilbene		D1	17	63–64	M-E, plates	85·8	8·6	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-2:2′-dimethylstilbene		D1	55	79–79·5	M, plates	85·7	8·3	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-2′-ethylstilbene	(9)	D1	80	78–79	M, then P, plates	85·9	8·5	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-2′-propylstilbene	(10)	D1	56	65	M, needles	86·3	8·8	86·0	8·8	$C_{19}H_{23}N$
4-dimethylamino-2′-*iso*propylstilbene	(11)	D1	73	54–55	M, sword-shaped needles	85·9	8·8	86·0	8·8	$C_{19}H_{23}N$
4-dimethylamino-2′-methyl-5′-*iso*propylstilbene		D1	28	75–76	M, plates	86·3	9·3	86·0	9·0	$C_{20}H_{25}N$

TABLE 16 (*cont.*)

compound	ref. no. to notes pp. 188–191	method of preparation	yield (%)	melting-point or boiling-point (°C)	solvent and appearance	found C	found H	calc. C	calc. H	formula
4-dimethylamino-2′:5′-diethylstilbene	(12)	D1	43	61	M, flattened needles	86·0	9·3	86·0	9·0	$C_{20}H_{25}N$
4-dimethylamino-2′:5′-dipropylstilbene	(13)	D1	29	66–67	M, flattened needles	86·2	9·8	86·0	9·5	$C_{22}H_{29}N$
4-dimethylamino-2′:5′-di*iso*propylstilbene	(14)	D1	poor	127–129	E or P, small prisms	86·0	9·6	86·0	9·5	$C_{22}H_{29}N$
4-dimethylamino-2′:5′-di*tert.*butylstilbene	(15)	D1	poor	117–119	M, needles	85·6	10·1	85·9	9·9	$C_{24}H_{23}N$
4-dimethylamino-2′:4′:6′-trimethylstilbene	(16)	D1	24	230–240/18 mm. 78	M, flattened needles	85·7	8·6	86·0	8·8	$C_{19}H_{22}N$
trans 2′-chloro-4-nitrostilbene	(17)	A1	30	123–124	M or dil. A, yellow needles	64·9	3·9	64·7	3·9	$C_{14}H_{10}O_2NCl$
trans 2′-chloro-4-aminostilbene		$SnCl_2$	86	86	M or B-P, buff needles	73·0	5·3	73·2	5·3	$C_{14}H_{12}NCl$
trans 2′-chloro-4-acetamidostilbene		—	—	165	B-P, buff plates	70·4	5·1	70·7	5·2	$C_{16}H_{14}ONCl$
2′-chloro-4-phenylazostilbene		—	good	145–146	A, orange plates	75·3	4·9	75·3	4·7	$C_{20}H_{15}NCl$
trans 2′-chloro-4-dimethylaminostilbene		E	30	105	M or P, thick greenish prisms	74·8	6·5	74·5	6·3	$C_{16}H_{16}NCl$
2-(2′-chlorophenyl)-1-(4′-dimethylaminophenyl)-ethanol		D	81	69–70	P, plates	69·7	6·7	69·7	6·6	$C_{16}H_{18}ONCl$
trans 2′-chloro-4-dimethylaminostilbene		D1	90	—	—	—	—	—	—	—
cis 2′-chloro-4-nitrostilbene	(18)	A2	—	82–83	M, pale yellow plates	64·7	3·9	64·7	3·8	$C_{14}H_{10}O_2NCl$
cis 2′-chloro-4-aminostilbene	(18)	$FeSO_4+NH_3$	—	100–105/0·01–0·1 mm. 41–42	pentane, plates	73·1	5·3	73·2	5·3	$C_{14}H_{12}NCl$
cis 2′-chloro-4-acetamidostilbene		—	—	134–135	A, needles	70·7	5·3	70·7	5·2	$C_{16}H_{14}ONCl$
cis 2′-chloro-4-dimethylaminostilbene		E	—	85	M, pale yellow plates	74·6	6·4	74·5	6·3	$C_{16}H_{16}NCl$
trans 3′-chloro-4-nitrostilbene	(19)	A2	—	118–119	E, yellow prisms	64·9	3·9	64·7	3·8	$C_{14}H_{10}O_2NCl$
trans 3′-chloro-4-aminostilbene		$SnCl_2$	75	142–143	B or B-P, needles	73·4	5·5	73·2	5·3	$C_{14}H_{12}NCl$
trans 3′-chloro-4-dimethylaminostilbene		D1	43	137–138	E, plates	74·7	6·2	74·5	6·3	$C_{16}H_{16}NCl$
4′-chloro-4-dimethylaminostilbene		D1	15	216	B-E, greenish plates	74·8	6·3	74·5	6·3	$C_{16}H_{16}NCl$
2-chloro-4-nitrostilbene		C	7	106–107	P (80–100°), clusters of yellow needles	64·8	4·7	64·5	4·3	$C_{14}H_{11}O_2NCl$
2-chloro-4-aminostilbene		$SnCl_2$	50	57–58	P (40–60°), clusters of pale cream needles	73·1	5·2	73·2	5·3	$C_{14}H_{12}NCl$
2-chloro-4-dimethylaminostilbene		E	50	106	M, plates	74·4	6·3	74·5	6·3	$C_{16}H_{16}NCl$
2′-bromo-4-dimethylaminostilbene		D1	7	107–108	E, yellow prisms	63·8	5·6	63·6	5·3	$C_{16}H_{16}NBr$
3′-bromo-4-dimethylaminostilbene		D1	12	145–146	B-E, yellowish prisms	64·0	5·4	63·6	5·3	$C_{16}H_{16}NBr$
trans 4′-bromo-4-nitrostilbene	(20)	A2	—	196–197	E	55·4	3·1	55·3	3·3	$C_{14}H_{10}O_2NBr$
cis 4′-bromo-4-nitrostilbene		A2	—	77	P	55·4	3·1	55·3	3·3	$C_{14}H_{10}O_2NBr$
trans 4′-bromo-4-aminostilbene		$SnCl_2$	68	210	B, buff plates	61·0	4·4	61·3	4·4	$C_{14}H_{12}NBr$
4′-bromo-4-dimethylaminostilbene	(21)	E	20	232–233	B, buff plates	63·9	5·5	63·6	5·3	$C_{16}H_{16}NBr$
2′-fluoro-4-dimethylaminostilbene	(22)	D1	33	124–125	E, plates	79·7	6·9	79·6	6·7	$C_{16}H_{16}NF$
4-amino-4′-hydroxystilbene		H_2+Raney Ni on nitro-cpd. in ethyl acetate soln. (23)	85	268–269 decomp.	ethyl acetate, brown plates	79·3	6·5	79·6	6·2	$C_{14}H_{13}ON$
4-amino-2′-methoxystilbene		$SnCl_2$ on nitro-cpd. (24)	40	76–77	M, needles	80·2	6·6	80·0	6·7	$C_{15}H_{15}ON$
4-dimethylamino-2′-methoxystilbene		E	60	89	P then M, plates	80·6	7·6	80·6	7·6	$C_{17}H_{19}ON$
4-nitro-4′-hydroxy-2′-methylstilbene		A1	mod.	162	dil. A	70·5	5·3	70·6	5·2	$C_{15}H_{13}O_3N$
4-amino-4′-hydroxy-2′-methylstilbene		H_2+Raney Ni in ethyl acetate	—	162	dil. M, orange needles B-P, pale orange plates	80·3	7·0	80·0	6·7	$C_{15}H_{15}ON$
trans 2:4-tetramethyldiaminostilbene	(25)	E	poor	oil	—	81·2	8·2	81·2	8·3	$C_{14}H_{14}N_2$
1-(3′-methoxyphenyl)-2-(4′-dimethylamino-phenyl)ethanol		D	26	74–75	P	74·9	7·7	75·0	7·8	$C_{17}H_{21}O_2N$
4-dimethylamino-3′-methoxystilbene		—	—	91–92	E, plates	80·3	7·4	80·6	7·5	$C_{17}H_{19}ON$
3′:4-tetramethyldiaminostilbene (from diamino, Ashley *et al.* 1942)		E	poor	120·5–121·5	M, yellow plates	81·2	8·7	81·2	8·3	$C_{14}H_{14}N_2$

4′-nitro-3-dimethylaminostilbene		A1	15	143–144	B-M,scarlet prisms	71·3	6·2	71·6	6·0	$C_{16}H_{16}O_2N_2$
4′-amino-3-dimethylaminostilbene		$SnCl_2$	poor	163–164	C, buff plates	80·5	7·5	80·6	7·6	$C_{16}H_{18}N_2$
4′-amino-4-dimethylaminostilbene		$SnCl_2$	60	173–174	dil. E, pale yellow plates	80·6	7·6	80·6	7·6	$C_{16}H_{18}N_2$
2:4-diacetamidostilbene		—	60	250–251	E, small plates	73·4	6·2	73·4	6·2	$C_{18}H_{18}O_2N_2$
2-amino-4-acetamidostilbene	(26)	$SnCl_2$	60	176–177	E, buff plates	76·1	6·4	76·2	6·4	$C_{16}H_{16}ON_2$
4-amino-2-acetamidostilbene	(27)	$SnCl_2$	60	146–147	dil. E, buff needles	76·0	6·5	76·2	6·4	$C_{16}H_{18}ON_2$
2-nitro-4-methylaminostilbene	(28)	—	60	107–108	M, purple needles	70·9	6·0	70·9	5·6	$C_{15}H_{14}O_2N_2$
2-amino-4-methylaminostilbene		$SnCl_2$	good	122–123	C, buff plates	80·7	7·6	80·7	7·2	$C_{15}H_{10}N_2$
2:4-dinitro-2′-methoxystilbene		B	33	176	ethyl acetate or A, orange plates	59·8	4·2	60·0	4·1	$C_{15}H_{15}O_5N_2$
2:4-diamino-2′-methoxystilbene		H_2+Raney Ni in ethyl acetate	—	152	ethyl acetate, golden brown leaflets	74·8	6·9	75·0	6·7	$C_{15}H_{16}ON_2$
1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene		D1	75	200–260/2 mm. 117	E, sulphur yellow plates with green fluorescence	87·9	7·1	87·9	7·0	$C_{20}H_{19}N$
1-(4′-dimethylaminophenyl)-2-(2′-naphthyl)ethylene		D1	poor	195	E, buff plates	87·7	7·0	87·9	7·0	$C_{20}H_{19}N$
1-(4′-dimethylaminophenyl)-2-(2′-methyl-1′-naphthyl)-ethylene		D1	poor	97	E, pale yellow prisms	87·9	7·7	87·7	7·3	$C_{21}H_{21}N$
2-phenyl-1-(4′-dimethylamino-1′-naphthyl)ethylene		D1	poor	227/3 mm. 68–69	E, flattened needles	87·5	7·2	87·9	7·0	$C_{20}H_{19}N$
1-phenyl-2-(2′-dimethylaminophenyl)propan-2-ol		D	60	64–65	needles	79·9	8·3	80·0	8·3	$C_{17}H_{21}ON$
2-dimethylamino-α-methylstilbene	(29)	Ac_2O	80	135–140/3 mm.	—	86·1	7·9	86·0	8·1	$C_{17}H_{19}N$
1-phenyl-2-(3′-dimethylaminophenyl)propan-2-ol		D	—	oil	—	79·7	8·6	80·0	8·3	$C_{17}H_{21}ON$
3-dimethylamino-α-methylstilbene		D1	good	oil	—	86·2	8·2	86·0	8·1	$C_{17}H_{19}N$
1-phenyl-2-(4′-dimethylaminophenyl)propan-2-ol		D	good	63–64	P, needles	80·0	8·4	80·0	8·3	$C_{17}H_{21}ON$
4-dimethylamino-α-methylstilbene		D1	good	92–93	P, plates	86·2	8·3	86·0	8·1	$C_{17}H_{19}N$
2-dimethylamino-α-ethylstilbene	(30)	D1	50	130–135/4 mm.	—	85·8	8·6	86·0	8·4	$C_{18}H_{21}N$
1-phenyl-2-(4′-dimethylaminophenyl)butan-2-ol		D	good	62–63	pentane, needles	80·3	8·8	80·3	8·6	$C_{18}H_{23}ON$
4-dimethylamino-α-ethylstilbene	(31)	P_2O_5	good	oil	—	86·2	8·7	86·0	8·4	$C_{18}H_{21}N$
4-dimethylamino-αβ-dimethylstilbene	(32)	D1	45	164–165/3·5 mm.	—	85·9	8·6	86·0	8·4	$C_{18}H_{21}N$
4-phenyl-3-(4′-dimethylaminophenyl)hexan-3-ol	(33)	D	—	200–204/3 mm.	—	81·1	9·2	80·7	9·2	$C_{20}H_{27}ON$
4-dimethylamino-αβ-diethylstilbene		D2	—	167–168/2 mm.	—	86·0	9·1	86·0	9·0	$C_{20}H_{25}N$
4-aminotolane	(34)	$SnCl_2$	70	131–132	P, light-brown needles	86·7	5·9	87·0	5·8	$C_{14}H_{11}N$
3-phenyl-1-(4′-dimethylaminophenyl)propan-1-ol		D	quant.	68–69	P, needles	79·6	8·5	80·0	8·3	$C_{17}H_{21}ON$
3-phenyl-1-(4′-dimethylaminophenyl)prop-1-ene		D2	good	51–52	pentane, needles	86·1	8·2	86·0	8·1	$C_{17}H_{19}N$
1-phenyl-3-(4′-dimethylaminophenyl)prop-1-ene	(35)	—	70	168/2·2 mm.	—	86·1	8·2	86·0	8·1	$C_{17}H_{19}N$
4′-dimethylamino-1:4-diphenylbutadiene	(36)	—	—	181	C, orange plates	86·5	7·7	86·7	7·7	$C_{18}H_{19}N$
1:1-diphenyl-2-(4′-dimethylaminophenyl)ethanol		D	66	126–127	C, then M, needles	83·4	7·2	83·2	7·3	$C_{22}H_{23}ON$
1:1-diphenyl-2-(4′-dimethylaminophenyl)ethylene		2*N* HCl at b.p.	—	124	E, pale yellow needles	87·9	7·1	88·2	7·1	$C_{22}H_{21}N$
1:2-diphenyl-2-(4′-dimethylaminophenyl)ethylene	(37)	—	—	oil	—	74·7	6·8	74·7	6·8	$C_{22}H_{21}N$, HCl, H_2O
1-phenyl-2:2-di-(4′-dimethylaminophenyl)ethylene	(38)	—	100	—	—	—	—	—	—	—
1:2-diphenyl-1-(4′-diethylaminophenyl)ethanol	(39)	D	—	oil	—	82·8	7·7 (on crude material)	83·5	7·7	$C_{24}H_{27}ON$
1:2-diphenyl-1-(4′-diethylaminophenyl)ethylene		D2	—	green oil	—	88·1	7·7	88·1	7·7	$C_{24}H_{25}N$
1-phenyl-2:2-di(4′-diethylaminophenyl)ethylene	(40)	D	—	—	—	84·5	8·5	84·4	8·6	$C_{28}H_{34}N$
1-phenyl-2:2-di(4′-diethylaminophenyl)ethylene dihydrochloride dihydrate		—	—	—	—	66·5	7·9	66·3	8·0	$C_{28}H_{34}N$, 2HCl, $2H_2O$
1-phenyl-2-(4′-dimethylaminophenyl)-2-(3′:4′-dimethoxyphenyl)ethanol	(41)	D	good	111–112	M, needles	76·6	7·3	76·4	7·2	$C_{24}H_{27}O_3N$
1-phenyl-2-(4′-dimethylaminophenyl)-2-(3′:4′-dimethoxyphenyl)ethylene		D2	good	104–105	M, clusters of needles	80·2	6·8	80·2	7·0	$C_{24}H_{25}O_2N$

(1) *o*-Dimethylaminobenzaldehyde was prepared by the method of Bamberger (1904).

(2) *m*-Dimethylaminobenzaldehyde was prepared by the method of Cocker, Harris & Loach (1938).

(3) The preparation of 2- and 3-nitrostilbenes by the same method has since been described by Bergmann & Schapiro (1947).

(4) This was prepared essentially as described by Sachs & Sachs (1905), a solution of 15 g. *p*-dimethylaminobenzaldehyde in 80 c.c. benzene being added to a mechanically stirred ethereal solution of benzyl magnesium chloride (from 15 g. of chloride and 3 g. magnesium in 100 c.c. ether). After warming 50 min. the mixture is decomposed with ammonium chloride (yield of carbinol 85%). It is dehydrated by warming for 20 min. with 5 vol. of acetic-hydrochloric acid (4:1), the yield of stilbene exceeding 80%. The *methiodide* crystallizes from much water, m.p. 220 to 225° C (Found: C, 55·9; H, 5·6. $C_{17}H_{20}NI$ requires C, 55·9; H, 5·5%). The *methosulphate* on the other hand is very soluble in water, fern-like aggregates of prisms from *iso*propanol, m.p. 203 to 210° C, giving slightly high values on analysis (Found: C, 62·4; H, 7·0. $C_{18}H_{23}O_4NS$ requires C, 61·9; H, 6·6%). The methosulphate was used to prepare other salts by double decomposition: of these, the methochloride was also very soluble in water and did not separate until 'salted out'; the methobromide and the methonitrate could be conveniently crystallized from hot water, whereas the methochlorate and methopersulphate were very sparingly soluble. The intense violet-blue fluorescence of the base disappears on salt formation, the salts displaying a faint pink or yellow fluorescence.

(5) Crude 4-iso*propylaminobenzaldehyde* was obtained in poor yield by the general method described in G.P., 105,105; Dippy, Hogarth, Watson & Williams (1937). The fraction b.p. 180 to 190° C/3 mm. consisted mainly of the desired compound (Found: C, 72·5; H, 8·0. $C_{10}H_{13}ON$ requires C, 73·6; H, 8·0).

(6) 4-*Diallylaminobenzaldehyde* was similarly obtained (8 g. from 25 g. diallylaniline) and formed an amber oil b.p. 190° C/15 mm. (Found: C, 77·5; H, 7·5. $C_{13}H_{15}ON$ requires C, 77·6; H, 7·4%). The *semicarbazone* had m.p. 184° C (Found: C, 64·9; H, 7·1. $C_{14}H_{18}ON_4$ requires C, 65·1; H, 7·0).

(7) 4-(*Ethyl-2'-acetoxyethylamino*)*benzaldehyde* was obtained by boiling the hydroxy-aldehyde (Dippy *et al.* 1937) with acetic anhydride, the fraction boiling above 200° C/1 mm. being the desired compound (Found: C, 66·2; H, 7·3. $C_{13}H_{17}O_3N$ requires C, 66·3; H, 7·3%).

(8) *vic. m*-Xylidine was separated from technical xylidine residues by a process based on that of Busch (1899), for the details of which and also for the raw material the authors are indebted to Dr E. H. Rodd of the Imperial Chemical Industries Ltd. The conversion of the base into 2-bromo-*m*-xylene (Jacobson & Deike 1887) was carried out by the method of Bigelow (1944) except that the diazonium solution was added to a cold solution of cuprous bromide in 48% hydrobromic acid and the mixture gradually heated. A Grignard reagent prepared from 56 g. of 2-bromo-*m*-xylene in ether was treated with 12 g. of paraformaldehyde previously dried in a vacuum over phosphoric oxide, the mixture being mechanically stirred for 48 hr. Dilute sulphuric acid was added, the ethereal layer separated, washed with water and alkali, dried over sodium sulphate, evaporated and distilled. The fraction b.p. 115 to 120° C/15 mm. solidified and could be crystallized from light petroleum, m.p. 82 to 82·5° C (yield 19 g.) (Found: C, 79·2; H, 8·8. $C_9H_{12}O$ requires C, 79·4; H, 8·9%). An ice-cold solution of 10 g. of the above alcohol in 10 g. of dimethylaniline was treated dropwise with 6·8 g. of thionyl chloride, the reaction being completed by warming for ½ hr. 2:6-*Dimethylbenzyl chloride* boiled at 100 to 105° C/16 mm. and solidified to a lachrymatory solid m.p. 27 to 30° C, which could not be recrystallized (Found: Cl, 23·6. $C_9H_{11}Cl$ requires Cl, 22·9%).

(9) Ethylbenzene was nitrated and the isomerides separated by distillation, as described by Cline & Reid (1927), but without employing a special still. The *o*-nitro-compound was reduced by shaking a solution in 10 volumes of alcohol with Raney nickel and hydrogen (compare Albert & Ritchie 1940), when a 95% yield of the base was obtained. This was converted into *o*-bromoethylbenzene in 42% yield using the method given for *o*-bromotoluene by Bigelow (1944). A Grignard reagent prepared from 47 g. of *o*-bromoethylbenzene and 7 g. of magnesium in 200 c.c. of ether was treated with gaseous formaldehyde obtained by the depolymerization of 16 g. of paraformaldehyde, previously dried over phosphoric oxide, in a stream of nitrogen. The mixture was finally refluxed for 20 min., cooled, decomposed with dilute sulphuric acid and distilled in steam. The distillate was saturated with salt and the carbinol extracted with ether, the extract washed with sodium hydroxide, dried and evaporated. On distillation 7 g. of ethylbenzene were recovered, and 12 g. of a fraction b.p. 115 to 120° C/17 mm. giving on redistillation 9 g. of carbinol b.p. 118 to 119° C/17 mm. As this did not give good analytical figures, it was analyzed as the 3:5-*dinitrobenzoate* m.p. 87 to 88° C (Found: C, 57·9; H, 4·6. $C_{16}H_{14}O_6N_2$ requires C, 58·2; H, 4·3%). 8·5 g. of the alcohol gave on treatment with diethylaniline and thionyl chloride, 7·5 g. of the *chloride* b.p. 98 to 100° C/15 mm. (Found: Cl, 22·2. $C_9H_{11}Cl$ requires Cl, 22·9%).

(10) Attempts to prepare *o*-bromopropylbenzene by the action of phosphorus pentabromide on *o*-propylphenol gave a very poor yield. Propylbenzene was therefore nitrated (Baddeley & Kenner 1935), 230 g. giving 82 g. of *o*-nitropropylbenzene b.p. 126 to 128° C/15 mm. and 145 g. of the *p*-isomeride b.p. 140 to 142° C/17 mm. Reduction of the *o*-compound with Raney nickel as above gave a 96% yield of the amine b.p. 109 to 111° C/16 mm. This afforded a 38% yield of *o*-bromopropylbenzene b.p. 92 to 94° C/14 mm., which was converted into the *o*-propylbenzyl alcohol as described above, the yield of fraction b.p. 125 to 127° C/13 mm. being 35%. It was characterized by the 3:5-*dinitrobenzoate*, plates from alcohol m.p. 86 to 87° C (Found: C, 59·0; H, 4·8. $C_{17}H_{16}O_6N_2$ requires C, 59·3; H, 4·7%). o-*Propylbenzyl chloride* was prepared

from the alcohol as described above in 75 % yield and boiled at 106 to 108° C/12 mm. (Found: Cl, 21·2. $C_{10}H_{13}Cl$ requires Cl, 21·1 %).

(11) *o*-Bromocumene could not be prepared from *p-iso*propylbenzoic acid. Cumene was prepared by the method of Meyer & Bernhauer (1929), the yield being as high as 50 %, in addition to some *p*-di*iso*propylbenzene. It gave a 91 % yield of 2:4-dinitrocumene when nitrated by the method used by Brady & Cunningham (1934) for propylbenzene. The partial reduction of the dinitrocumene, also using the procedure of Brady & Cunningham, afforded a 65 % yield of 2-*nitro*-4-*aminocumene*, crystallizing from benzene in yellow plates m.p. 52 to 53° C (Found: C, 60·2; H, 6·8. $C_9H_{12}O_2N_2$ requires C, 60·0; H, 6·7 %). The *acetyl* compound formed flattened needles from benzene-petroleum, m.p. 117 to 118° C (Found: C, 59·5; H, 6·5. $C_{11}H_{14}O_3N_2$ requires C, 59·5; H, 6·4 %). Deamination of the base by the hypophosphorous acid method as described by Kornblum (1944) gave a 75 to 80 % yield of *o*-nitrocumene b.p. 107 to 108° C/10 mm. Reduction of this with Raney nickel gave a 90 % yield of *o*-cumidine, from which a 56 % yield of *o*-bromocumene was obtained by the method already described. 64 g. of bromocumene gave 17·5 g. (38 %) of a fraction b.p. 110 to 111° C/9 mm. consisting mainly of o-iso*propylbenzyl alcohol* (Found: C, 79·0; H, 9·3. $C_{10}H_{14}O$ requires C, 80·0; H, 9·4 %); it was characterized by the 3:5-*dinitrobenzoate*, forming needles from dilute methyl alcohol, m.p. 83 to 84° C (Found: C, 59·5; H, 4·7. $C_{17}H_{16}O_6N_2$ requires C, 59·3; H, 4·7 %). o-iso*Propylbenzyl chloride* formed from the above alcohol in good yield had b.p. 91° C/9 mm. (Found: Cl, 21·0. $C_{10}H_{13}Cl$ requires Cl, 21·1 %).

(12) 32 g. of *p*-diethylbenzene, 32 g. of chloromethyl ether (compare Fuson & McKeever 1942) and 80 c.c. of carbon disulphide were stirred at 0° C while 19 g. of stannic chloride were dropped in in the course of an hour. After a further hour's stirring the mixture was poured on to ice and the 2:5-*diethylbenzyl chloride* extracted with ether; 30 g. boiled at 130 to 135° C/23 mm. and gave on refractionation 27 g. b.p. 132 to 133° C/23 mm. (Found: Cl, 18·8. $C_{11}H_{13}Cl$ requires Cl, 19·4 %).

(13) Chloromethylation of 40 g. of *p*-dipropylbenzene as above gave 23 g. of 2:5-*dipropylbenzyl chloride* b.p. 150 to 151° C/25 mm. (Found: Cl, 16·0. $C_{13}H_{19}Cl$ requires Cl, 16·9 %).

(14) 30 g. of *p*-di*iso*propylbenzene were chloromethylated as above giving 11·5 g. of 2:5-*di*iso*propylbenzyl chloride*, b.p. 146 to 152° C/20 mm. (Found: Cl, 16·9. $C_{13}H_{19}Cl$ requires Cl, 16·9 %).

(15) The chloromethylation of *p*-di*tert.*butylbenzene (conveniently prepared by the method of Meyer & Bernhauer 1929) at 0° C as described above gave unchanged starting material and was repeated at room temperature. After several fractionations 5 g. of a fraction b.p. 150 to 155° C/23 mm. were obtained from 30 g. of the hydrocarbon, but this still gave low values for chlorine (Found: Cl, 11·0, 11·2. $C_{15}H_{23}Cl$ requires Cl, 14·9 %).

(16) A neutral by-product was isolated in considerable quantity and consisted of $\alpha\beta$-dimesitylethane m.p. 116 to 117° C (Wenzel 1914 gives 117 to 118° C) (Found: C, 90·1; H, 9·7. Calc. C, 90·2; H, 9·8 %).

(17) Temperature must be kept below 140° C.

(18) 9·05 g. of *p*-nitrophenylacetic acid were converted into the potassium salt in methyl alcohol solution, this evaporated to dryness and the residue dried. It was boiled for 3½ hr. (oil bath at 180° C) with 7·0 g. of *o*-chlorobenzaldehyde, 2·5 g. anhydrous potassium carbonate and 15 c.c. of acetic anhydride, then cooled and treated with water. The precipitated semisolid mass was repeatedly extracted with hot 2*N* sodium hydroxide, giving on acidification 12·1 g. of crude acid. A small amount of neutral solid remained which proved to be *trans* 2′-chloro-4-nitrostilbene. The acid product was crystallized from acetic acid, giving a less soluble acid, foliated prisms m.p. 214° C after repeated crystallization (Found: C, 59·1; H, 3·3. $C_{15}H_{10}O_4NCl$ requires C, 59·3; H, 3·3 %), presumably the *cis* form of 2′-*chloro*-2-(4″-*nitrophenyl*)*cinnamic acid*, and a more soluble *isomeride*, needles m.p. 163 to 164° C (Found: C, 59·5; H, 3·4); there was also a small amount of a third acid m.p. 212 to 213° C which was identified as *o*-chlorocinnamic acid (Found: C, 58·8; H, 4·0. Calc. C, 59·2; H, 3·8 %). In a larger run 113 g. of nitrophenylacetic acid gave, after a reaction time of 2 hr., 11 g. of stilbene (7·5 %), 1 to 2 g. of chlorocinnamic acid and 140 g. of almost pure *cis* chloronitrophenylcinnamic acid (82 %). The two isomerides were found in comparable amounts in the earlier runs.

15 g. of the *cis* acid were heated to 220° C for 45 min. with 80 c.c. of quinoline and 1·5 g. copper chromite catalyst, then poured into excess of dilute hydrochloric acid (1:2). The dark solid was dissolved in ether, filtered from the catalyst, freed from unchanged acid by extraction with sodium hydroxide (2·4 g. recovered), the solution dried and evaporated. The residue on crystallization from methyl alcohol (charcoal) gave several crops; the main bulk formed pale yellow plates m.p. 82 to 83° C consisting of the cis *stilbene*. The second constituent, though present in smaller amount, was much less soluble and formed fine long needles m.p. 123 to 124° C, identical with the *trans* stilbene, together with some intermediate fractions. In a large run, when several small-scale experiments were worked up together, there was obtained from a total of 140 g. of acid 26 g. of *cis* and 8 g. of *trans* stilbene with 37 g. of a mixture of the two.

The acid of m.p. 163 to 164° C was decarboxylated in a similar manner and gave a poor yield of pure *trans* stilbene. 0·75 g. of *cis* stilbene were boiled for 15 min. with 7·5 c.c. of nitrobenzene and a crystal of iodine, the solvent being then distilled off under reduced pressure. The residue was the pure *trans* stilbene, m.p. and mixed m.p. 123 to 124° C after crystallization.

The above *cis* stilbene (3 g.) in 50 c.c. of alcohol was added to a well-shaken solution of 33 g. of ferrous sulphate in 50 c.c. of water and 60 c.c. of concentrated ammonia, the mixture being heated on the steam

bath for an hour with frequent shaking. After cooling, the *base* was repeatedly extracted with petroleum (b.p. 60 to 80° C). The extract was dried, percolated through a short column of alumina and evaporated, the residue being distilled in a high vacuum at 100 to 105° C/0·01 to 0·1 mm.; it solidified.

(19) *m*-Chlorobenzaldehyde was condensed with potassium *p*-nitrophenylacetate exactly as described for the *ortho* isomeride. The reaction was exothermic and was completed by heating to 180° C for 2 hr. The yield of 3′-*chloro*-2-(4″-*nitrophenyl*)*cinnamic acid* was 16·5 g. from 20 g. of the aldehyde and only one isomeride appeared to be present. It crystallized from acetic acid in needles m.p. 203 to 204° C (Found: 59·2; H, 3·3. $C_{15}H_{10}O_4NCl$ requires C, 59·3; H, 3·3 %). It was accompanied by a small amount of neutral material, evidently trans 3′-*chloro*-4-*nitrostilbene*, which formed yellow prisms from alcohol, m.p. 118 to 119° C (Found: C, 64·9; H, 3·9. $C_{14}H_{10}O_2NCl$ requires C, 64·7; H, 3·9 %). The above acid was decarboxylated as described in note (18); all fractions of the product, obtained in excellent yield, had m.p. and mixed m.p. 118 to 119° C and were thus the *trans* stilbene.

(20) *p*-Bromobenzyl magnesium bromide could not be condensed with *p*-dimethylaminobenzaldehyde by the method successfully used for the *o*- and *m*-bromobenzyl bromides. 56 g. of *p*-bromobenzaldehyde were condensed with potassium *p*-nitrophenylacetate and gave 15 g. of 4′-*bromo*-2-(4″-*nitrophenyl*)*cinnamic acid*, cream needles from acetic acid m.p. 232 to 233° C (Found: C, 51·9; H, 3·0. $C_{15}H_{10}O_4NBr$ requires C, 51·7; H, 2·9 %). 15 g. of the above acid gave 8 g. of the *trans* stilbene m.p. 196 to 197° C; the mother liquors from this contained a small amount of an *isomeride* m.p. 77° C after crystallization from petroleum, presumably the *cis* compound (Found: C, 55·4; H, 3·1. $C_{14}H_{10}O_2NBr$ requires C, 55·3; H, 3·3 %).

(21) This compound could not be obtained by method D.

(22) *o*-Fluorobenzyl bromide was prepared by the bromination of *o*-fluorotoluene using the method of Schmid & Karrer (1946).

(23) Cullinane (1923).

(24) Pfeiffer (1915); catalytic reduction in presence of Raney nickel gives an oil, evidently 4-*amino*-2′-*methoxydiphenylmethane* (Found: C, 79·0; H, 7·7. $C_{15}H_{17}ON$ requires C, 79·3; H, 7·5 %).

(25) The diaminostilbenes were prepared as described by Ruggli & Dinger (1941) but pure compounds were not obtained by catalytic reduction with Raney nickel. Ferrous sulphate and ammonia were satisfactory.

(26) 2-Nitro-4-aminostilbene (Thiele & Escales 1901) forms a *diacetyl* compound when boiled with acetic anhydride for an hour, yellow needles from methyl alcohol m.p. 147° C (Found: C, 66·6; H, 5·0. $C_{18}H_{16}O_4N_2$ requires C, 66·7; H, 5·0 %), the orange monoacetyl compound m.p. 192 to 193° C being formed as a by-product; it becomes the sole product if the amine is merely dissolved in acetic anhydride or when acetyl chloride is the acetylating agent used. Attempts to reduce the monoacetyl compound with hydrogen in the presence of Raney nickel gave a non-basic orange-brown compound m.p. 170 to 172° C which may be the azoxy-compound.

(27) 4-Nitro-2-aminostilbene (Thiele & Escales 1901) also forms a *diacetyl* compound on boiling with acetic anhydride, forming long straw-coloured needles from acetic acid m.p. 194 to 195° C (Found: 66·6; H, 5·0. $C_{18}H_{16}O_4N_2$ requires C, 66·7; H, 5·0 %), whereas Thiele & Escales's monoacetyl compound m.p. 220° C is produced by short warming with acetic anhydride or when acetyl chloride is used; it is also formed by boiling 2·3 g. of the diacetyl compound for 30 min. with 25 c.c. of 20 % sodium hydroxide and 50 c.c. of alcohol. Hydrogenation of the monoacetyl compound in ethyl acetate in the presence of Raney nickel proceeded very rapidly and was complete in about 1 hr. The solution on concentration deposited a pale buff material of fibrous appearance, forming long needles from methyl alcohol, m.p. 163° C, evidently 4-*amino*-2-*acetamidodiphenylethane* (Found: C, 75·6; H, 7·2. $C_{16}H_{18}ON_2$ requires C, 75·6; H, 7·1 %); it is acetylated to the diacetyl compound, long silky needles m.p. 228 to 229° C depressed by the admixture of 2:4-diacetamidostilbene. Reduction with stannous chloride was satisfactory but both this compound and its isomeride are difficult to obtain free from the last traces of tin salts.

(28) 2-*Amino*-4-*methylaminostilbene*. 3 g. of 2-nitro-4-aminostilbene were dissolved in 10 c.c. of hot methyl sulphate and left overnight. The excess of reagent was destroyed with 10 % alkali and the dark oil obtained was taken up in methyl alcohol, from which purple needles of 2-*nitro*-4-*methylaminostilbene* gradually separated, m.p. 107 to 108° C (Found: C, 70·9; H, 6·0. $C_{15}H_{14}O_2N_2$ requires C, 70·9; H, 5·6 %); the same compound is also formed under milder conditions and the formation of a dimethylamino-compound was not observed under any of the conditions tried. The acetyl compound of the amine formed soft needles from dilute alcohol, m.p. 145 to 147° C depressed by admixture of 4-amino-2-acetamidostilbene.

Attempts to prepare the isomeric 4-amino-2-methylaminostilbene and also the 2-dimethylamino-compound have not yet been successful, although the corresponding nitro-compounds are known (Neber, Knöller, Herbst & Trisler 1929).

(29) Dehydration with acetic-hydrochloric acid and with phosphoric oxide failed.

(30) *o*-Aminopropiophenone (Elson, Gibson & Johnson 1930) was methylated as described by Bogert & Nabenhauer (1924) giving its own weight of o-*dimethylaminopropiophenone* b.p. 128 to 132° C/22 mm. (Found: C, 74·2; H, 8·8. $C_{11}H_{15}ON$ requires C, 74·5; H, 8·6 %); the picrate formed yellow needles m.p. 155 to 156° C. The ketone was used for the above preparation.

(31) p-*Dimethylaminopropiophenone* was prepared in 50 % yield as above, long needles from petroleum m.p. 102 to 103° C (Found: C, 74·6; H, 8·6. $C_{11}H_{15}ON$ requires C, 74·5; H, 8·6 %); the *oxime* crystallized from benzene-petroleum, m.p. 162 to 163° C (Found: C, 69·0; H, 8·4. $C_{11}H_{16}ON_2$ requires C, 68·7; H, 8·4 %).

(32) 10 g. of *β*-4-dimethylaminodeoxybenzoin (Jenkins, Buck & Bigelow 1930) were added to a solution of 2 g. of potassium in 40 c.c. of *tert.*butyl alcohol, the solution cooled and treated with 10 c.c. of methyl iodide, then warmed for an hour. 2-*Phenyl*-1-(4′-*dimethylaminophenyl*)*propan*-1-*one* (10 g.) crystallized from dilute methanol in plates, m.p. 120 to 121° C (Found: C, 80·7; H, 7·6. $C_{17}H_{19}ON$ requires C, 80·6; H, 7·6 %).

(33) 2-*Phenyl*-1-(4′-*dimethylaminophenyl*)*butan*-1-*one* was prepared exactly like the lower homologue, plates from dilute ethanol m.p. 108 to 109° C (Found: C, 80·9; H, 8·0. $C_{18}H_{21}ON$ requires C, 80·9; H, 7·9 %).

(34) A suspension of 4-nitrostilbene dibromide (Pfeiffer & Eistert 1930) in 30 c.c. of boiling alcohol was treated with a solution of 5·4 g. of potassium hydroxide in 30 c.c. of methanol in the course of 15 min., the mixture being then boiled under reflux for a further 30 min. Much water was added, the mixture acidified and extracted with benzene, the extract washed, dried and percolated through a short column of alumina. The solid recovered on evaporation was recrystallized from methanol giving 2 g. of 4-*nitrotolane*, pale yellow needles m.p. 129 to 130° C (Found: C, 75·2; H, 4·4. $C_{14}H_9ON_2$ requires C, 75·4; H, 4·1 %).

(35) 1·5 g. 4′-dimethylaminochalcone (Fecht 1907), 4·5 c.c. 100 % hydrazine hydrate and 32 c.c. of a 5 % solution of sodium in alcohol were heated in a sealed tube to 200° C for 16 hr.

(36) Prepared from pure *p*-dimethylaminocinnamaldehyde (König, Schramek & Rösch 1928), the compound melts at 181° C and not 171° C as found by Sachs & Weigert (1907).

(37) By dehydration of the corresponding alcohol (Busignies 1909). It was analyzed as the *hydrochloride monohydrate*, feathery needles from methanol—HCl, m.p. 174 to 175° C (decomp.).

(38) The carbinol (Madelung & Völker 1926) was quantitatively dehydrated on warming for 10 min. with 8 vol. of 1:1 pyridine—acetic anhydride, a transient blue colour being developed.

(39) *p*-Dimethylaminobenzophenone could not be obtained by the method of Shah & Chaubal (1932), but the procedure described by Hurd & Webb (1944) for the lower homologue gave a good yield of the ketone.

(40) *pp′*-Tetraethyldiaminobenzophenone gave on treatment with benzyl magnesium iodide its own weight of carbinol which could not be purified and on distillation at 1 mm. underwent dehydration. The ethylene was purified by precipitating the *dihydrochloride dihydrate*, from which it could be regenerated as a gum.

(41) 4′-*Dimethylamino*-3:4-*dimethoxybenzophenone* was prepared in 63 % yield by Hurd & Webb's procedure (1944) from 3:4-dimethoxybenzanilide and crystallized from benzene-petroleum in yellow rhombs m.p. 119 to 120° C. Attempts to prepare it by Shah & Chaubal's (1932) method were unsuccessful. It did not form an oxime (Found: C, 71·1; H, 6·7. $C_{17}H_{19}O_3N$ requires C, 71·6; H, 6·7 %).

Other compounds

5-*Styrylacridine*. 4 g. each of benzaldehyde, 5-methylacridine and zinc chloride were heated under reflux for 6 hr. The cooled mass was extracted with ammonia and washed with alcohol, then recrystallized from alcohol with a little benzene, forming stout yellow prisms m.p. 182° C (Found: C, 89·5; H, 5·6. $C_{21}H_{15}N$ requires C, 89·7; H, 5·4 %).

2-(2′-*Dimethylaminophenyl*)*naphthalene*. 2-(2′-Nitrophenyl)naphthalene, prepared as described by Elks, Haworth & Hey (1940), was quantitatively reduced to the amine by means of hydrogen and Raney nickel in alcoholic solution. Methylation by process E gave the new *base* which was obtained as an oil. It was converted into the picrate which melted at 203 to 204° C after crystallization from toluene. The base regenerated from it by means of sodium carbonate and ether boiled at 172 to 174° C/1 mm. and solidified to a mass of feathery needles m.p. 53 to 54° C (Found: C, 87·3; H, 6·8. $C_{18}H_{17}N$ requires C, 87·4; H, 6·9 %).

2-(4′-*Dimethylaminophenyl*)*naphthalene*. The 4-amino compound was again obtained from the nitro by reduction with Raney nickel and hydrogen and on methylation gave the desired *base*, which was purified by chromatography. It crystallized from methyl alcohol or from *cyclo*hexane in buff plates m.p. 129° C (Found: C, 87·7; H, 6·7. $C_{18}H_{17}N$ requires C, 87·4; H, 6·9 %).

4-*Methylaminodiphenyl ether and* 4-*dimethylaminodiphenyl ether*. 4-Aminodiphenyl ether was prepared by the reduction of the nitro-compound with hydrogen and Raney nickel. After methylation by process E a mixture of products was obtained from which some of the original amine m.p. 85° C separated in solid form. The remaining liquid was converted into the picrate, which separated in two approximately equal crops. The first was recrystallized from benzene-alcohol, then twice from pure alcohol and melted at 136 to 137° C. It was the *picrate* of the dimethyl compound (Found: C, 54·1; H, 4·1. $C_{20}H_{18}O_8N_4$ requires C, 54·3; H, 4·1 %). The second *picrate* was much more soluble in alcohol and less so in benzene and was recrystallized from benzene-alcohol, m.p. 160° C (Found: C, 53·4; H, 3·7. $C_{19}H_{16}O_8N_4$ requires C, 53·3; H, 3·8 %). The *monomethyl* ether regenerated from it by passing a benzene solution over activated alumina crystallized from petroleum (b.p. 60 to 80° C) in prisms m.p. 57 to 58° C (Found: C, 78·6, 78·5; H, 6·6, 6·7. $C_{13}H_{13}ON$ requires C, 78·4; H, 6·6 %). The *dimethyl* ether similarly obtained from the picrate m.p. 137 to 138° C was a yellow oil b.p. 150° C/1 mm. (Found: C, 78·5; H, 6·8. $C_{14}H_{15}ON$ requires C, 78·8; H, 7·1 %).

4-*Dimethylaminodiphenyl sulphide.* The amino-compound (best prepared by the catalytic reduction of the nitro-compound with hydrogen and Raney nickel) was methylated by process E and after purification by chromatography crystallized from petroleum (b.p. 60 to 80° C) in clusters of needles m.p. 67° C (Found: C, 73·3; C, 6·6. $C_{14}H_{15}NS$ requires C, 73·3; H, 6·6 %).

p-*Nitrophenyl* o-*tolyl sulphide.* 12·4 g. of *o*-thiocresol were added to 5·6 g. of potassium hydroxide and 1 c.c. of water, followed by 18·7 g. of *p*-chloronitrobenzene, the mixture being heated under reflux to 150 to 160° C for 3 hr. The cooled mass was extracted with ether, the extract washed with alkali then water, dried and evaporated. The residue after two distillations gave 16 g. of the *sulphide* b.p. 187 to 189° C/2 mm., which solidified and formed pale yellow plates from methyl alcohol m.p. 66° C (Found: C, 63·5; H, 4·4. $C_{13}H_{11}O_2NS$ requires C, 63·6; H, 4·5 %).

p-*Aminophenyl* o-*tolyl sulphide.* 11·7 g. of the above compound in 200 c.c. of methyl alcohol were shaken in an atmosphere of hydrogen with 1 g. of Raney nickel until absorption ceased (3·2 l.). The catalyst was filtered off and the solution evaporated under reduced pressure, the residue taken up in ether, the amine extracted with dilute acid, the acid solution basified and the amine again extracted with ether. On evaporation of the dried extract 9 g. of solid were recovered, clusters of needles m.p. 53° C after two crystallizations from petroleum (b.p. 40 to 60° C) (Found: C, 72·4; H, 6·1. $C_{13}H_{13}NS$ requires C, 72·5; H, 6·1 %).

p-*Dimethylaminophenyl* o-*tolyl sulphide.* Methylation of the above amine by process E gave a moderate yield of the *dimethyl* compound, which was chromatographed in petroleum solution, then recrystallized twice from methyl alcohol, forming stout pale yellow needles m.p. 102° C (Found: C, 73·9; H, 7·3. $C_{15}H_{17}NS$ requires C, 74·0; H, 7·1 %).

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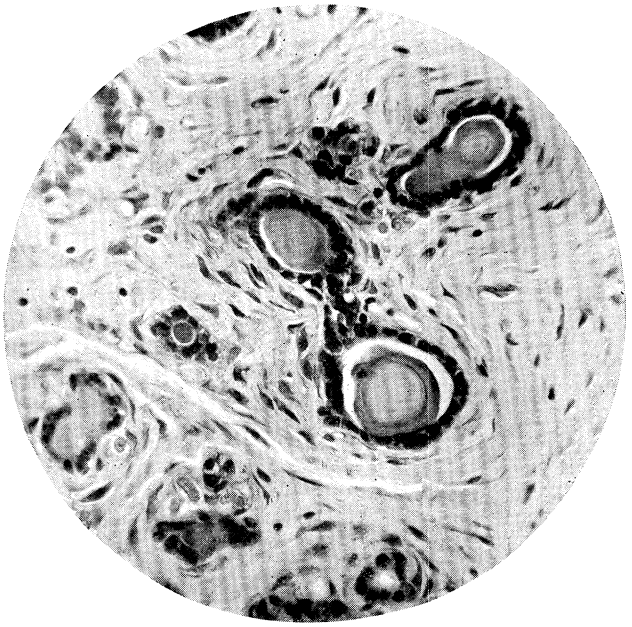

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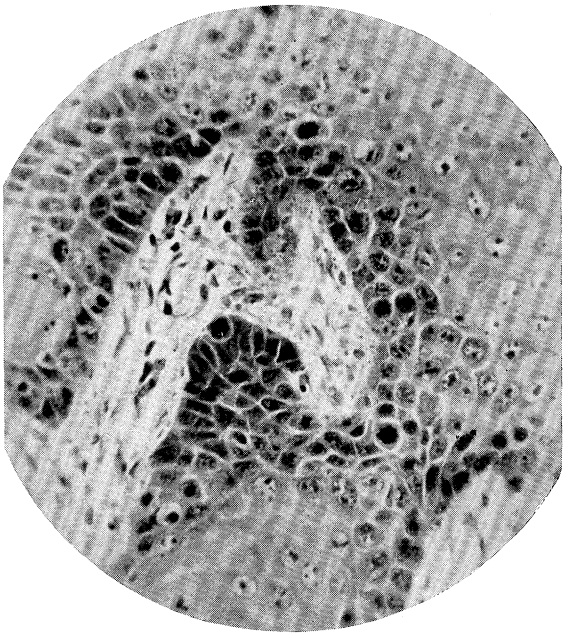

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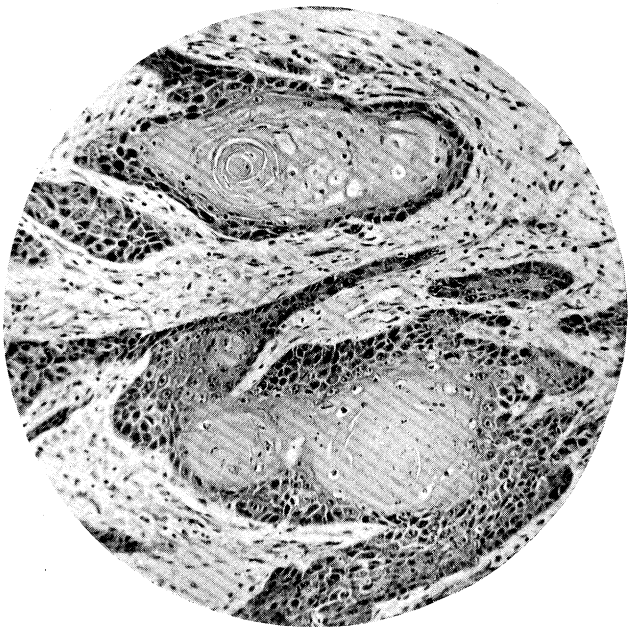

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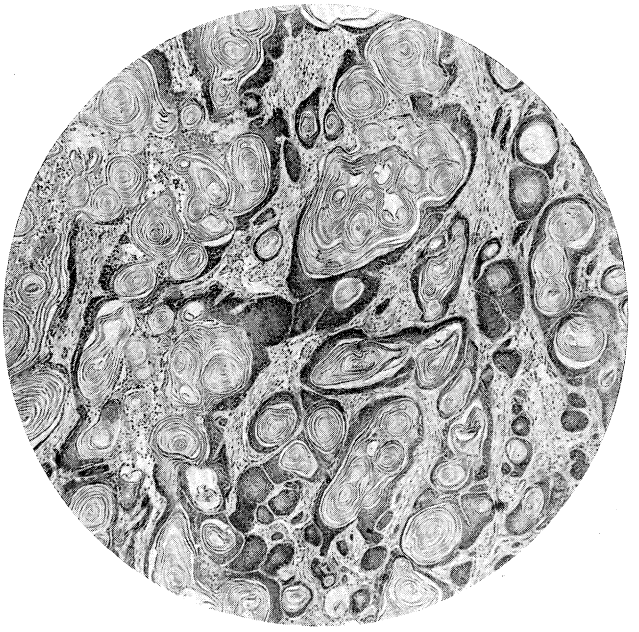

FIGURE 11

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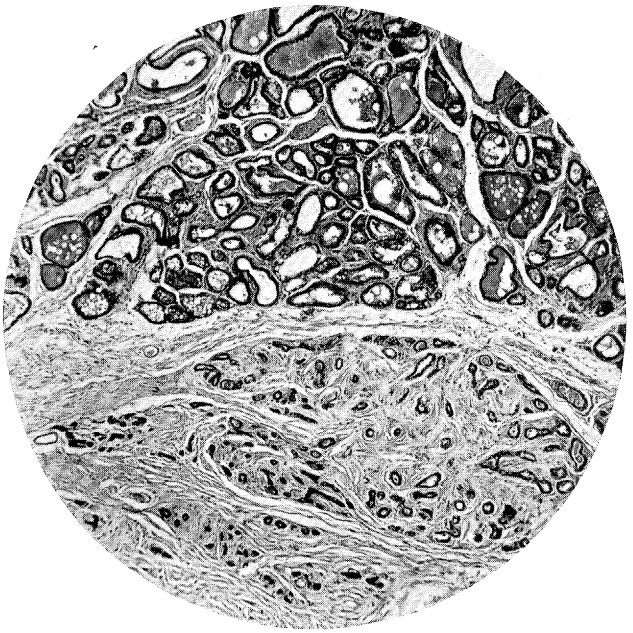

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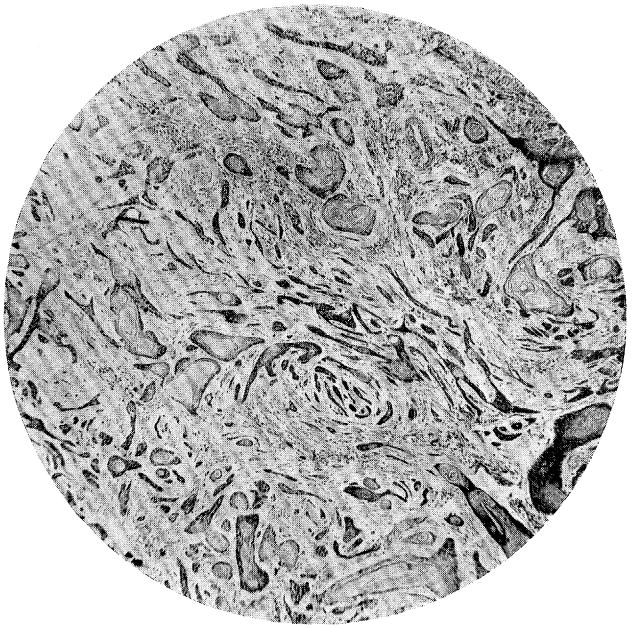

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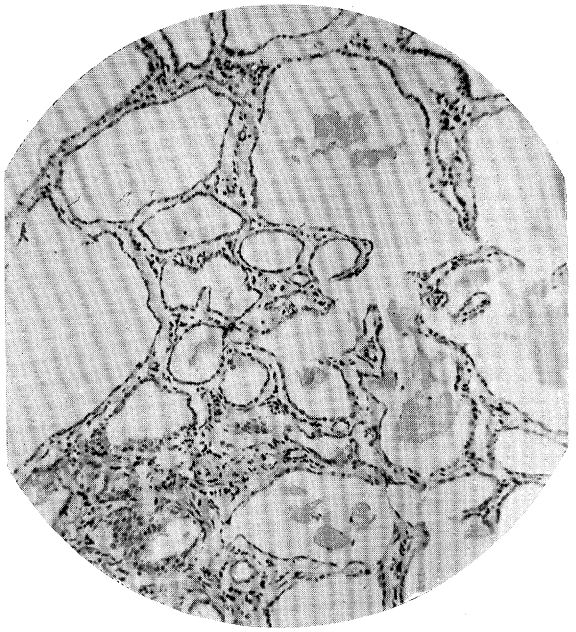

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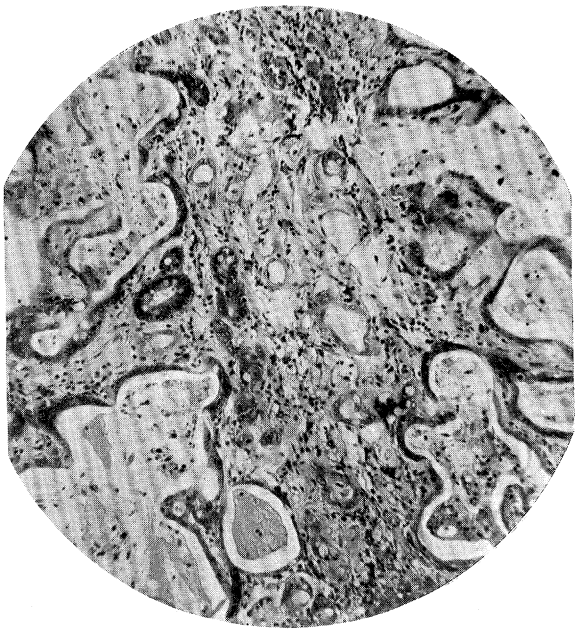

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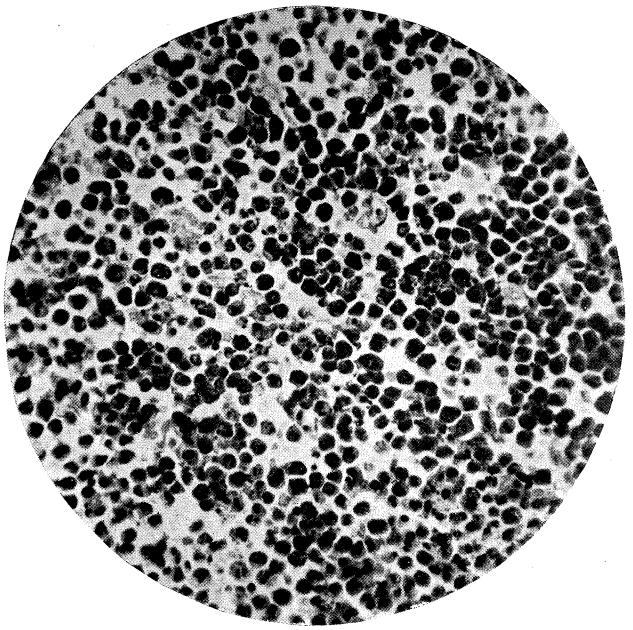

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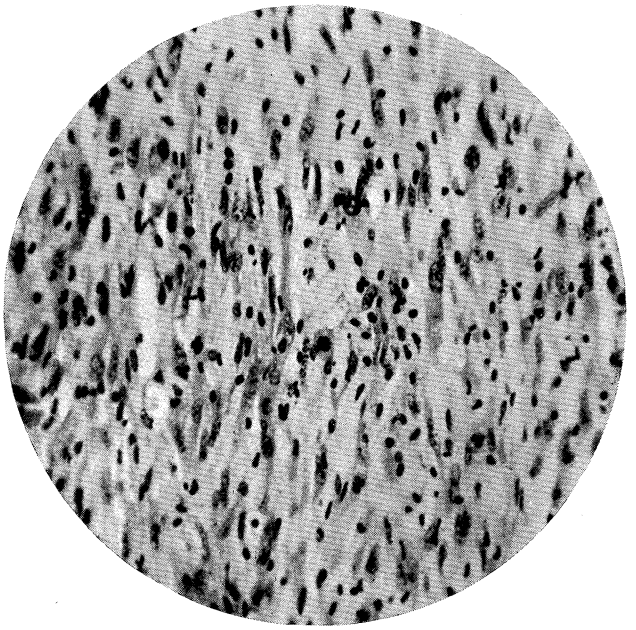

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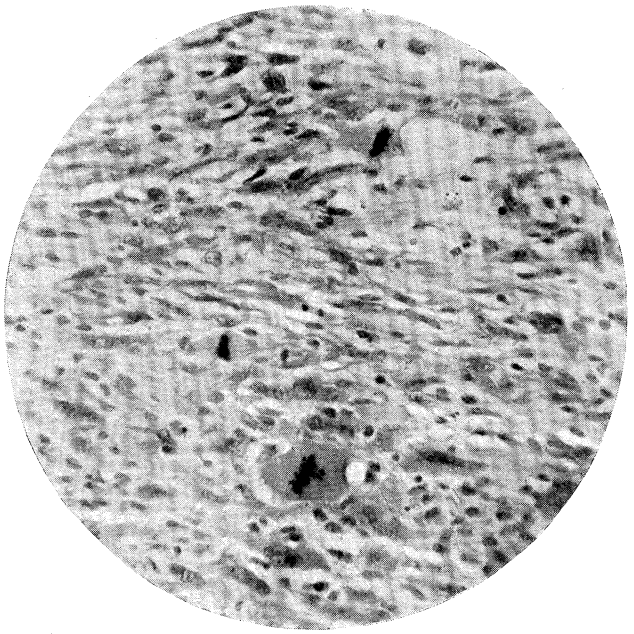

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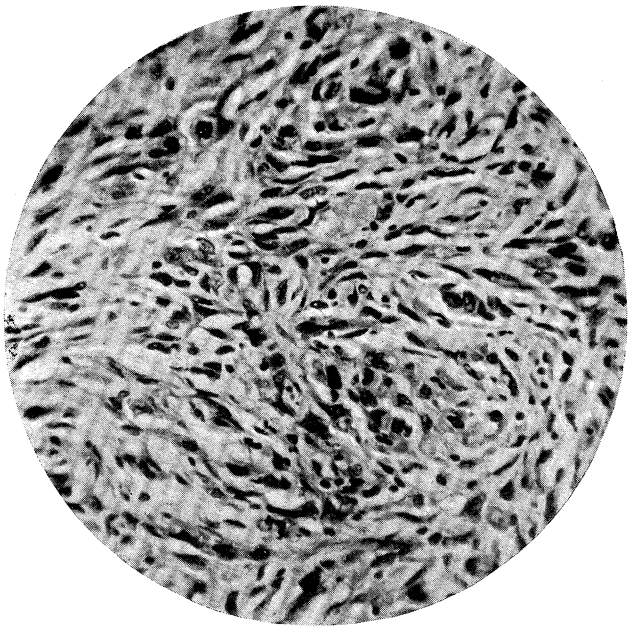


FIGURE 19

Description of Plates 2 to 4.

Plate 2

Figure 8. Mammary adenoma induced by 4-aminostilbene (experiment 122).

Figure 9. Squamous carcinoma of external acoustic duct induced by 4-diethylaminostilbene (experiment 125).

Figure 10. Squamous cell carcinoma of the external acoustic duct following subcutaneous injection (in the flank) of 4-diethylaminostilbene (experiment 125).

Figure 11. Keratinizing squamous cell carcinoma of external acoustic duct induced by 4-diethylaminostilbene (experiment 125).

Plate 3

Figure 12. Mammary adenoma induced by 4-dimethylaminostilbene (experiment 128).

Figure 13. Squamous cell carcinoma of external acoustic meatus induced by 4-dimethylaminostilbene (experiment 128).

Figure 14. Cholangiectasis induced by 4-dimethylaminostilbene (experiment 128).

Figure 15. Cholangioma induced by 4-dimethylaminostilbene (experiment 128).

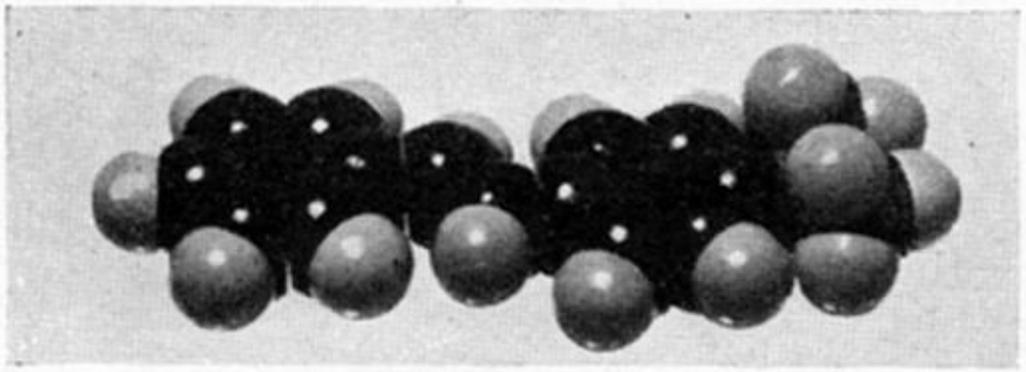
Plate 4

Figure 16. Lymphosarcoma induced by 4-dimethylaminostilbene (experiment 128).

Figure 17. Spindle-cell sarcoma induced by 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene (experiment 132).

Figure 18. Sarcoma induced by 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene (experiment 132).

Figure 19. Sarcoma induced by 1-(4′-dimethylaminophenyl)-2-(1′-naphthyl)ethylene (experiment 132).



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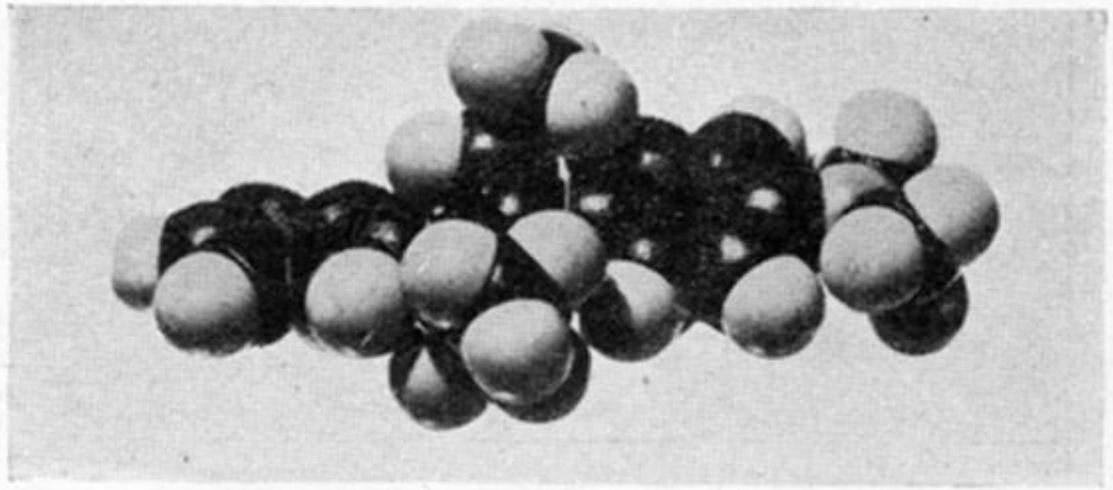

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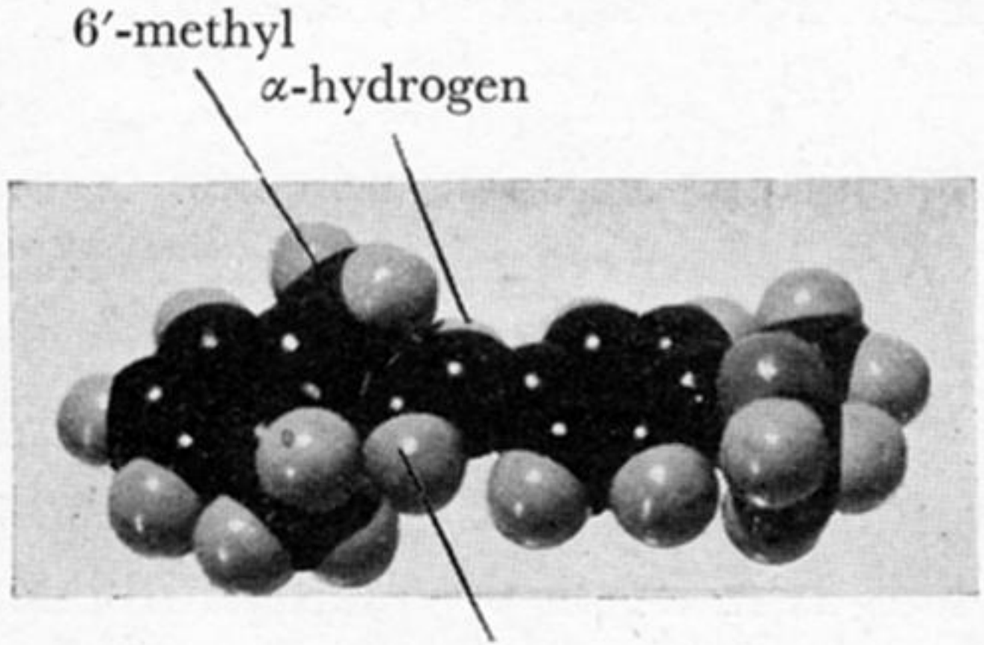

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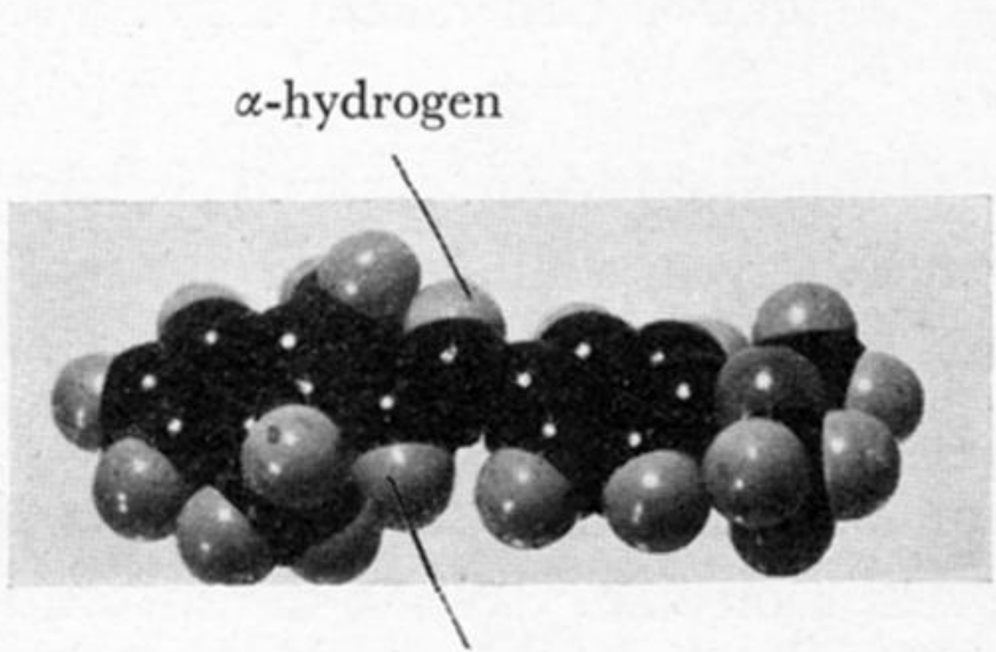
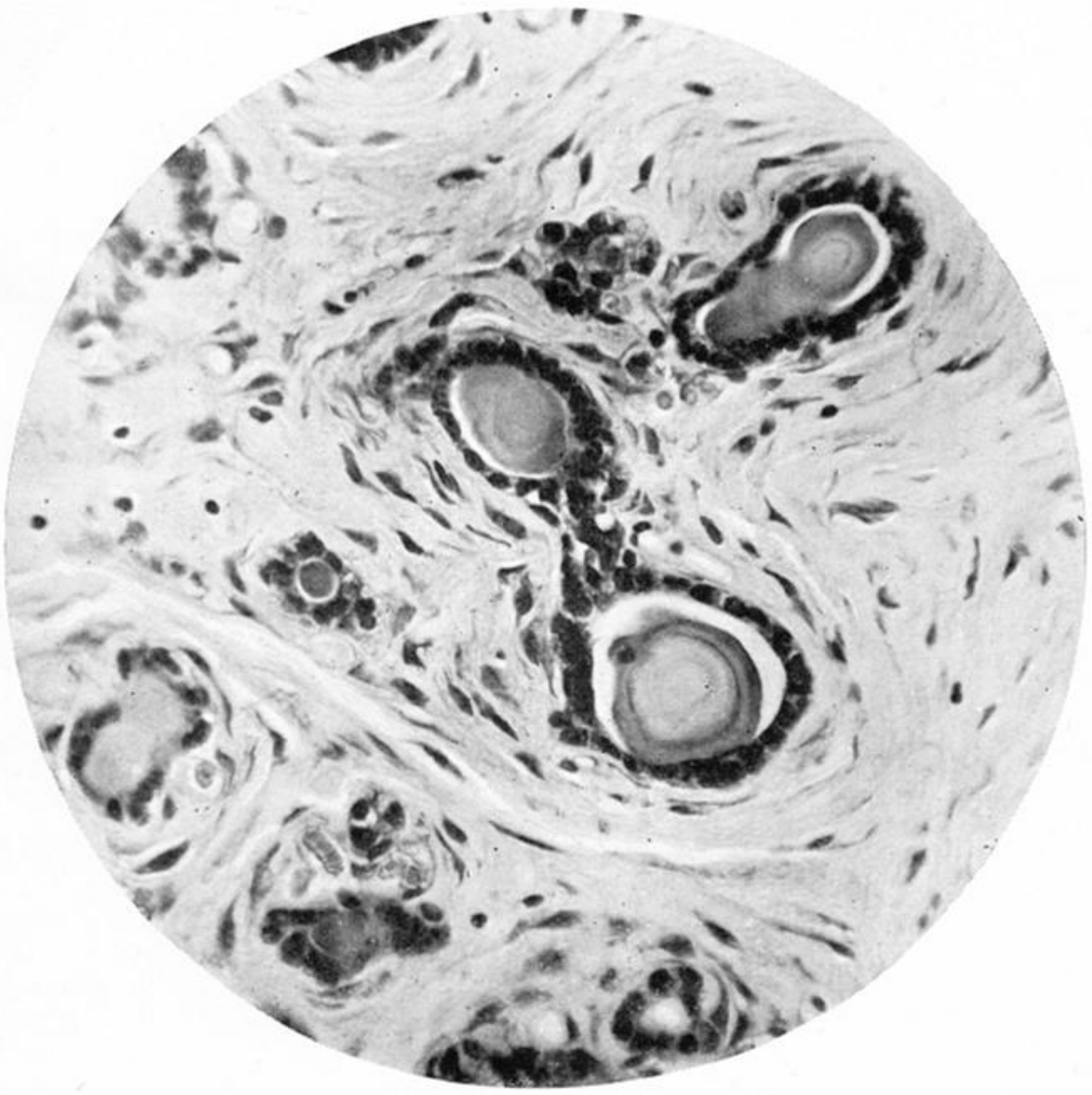


D

FIGURE 7. Fisher-Hirschfelder-Taylor molecular models to show: A, the planar character of 4-dimethylaminostilbene; B, the lack of planarity in 4-dimethylamino-$\alpha\beta$-diethylstilbene; C and D, possible planar and non-planar forms respectively of 4-dimethylamino-2′:6′-dimethylstilbene.



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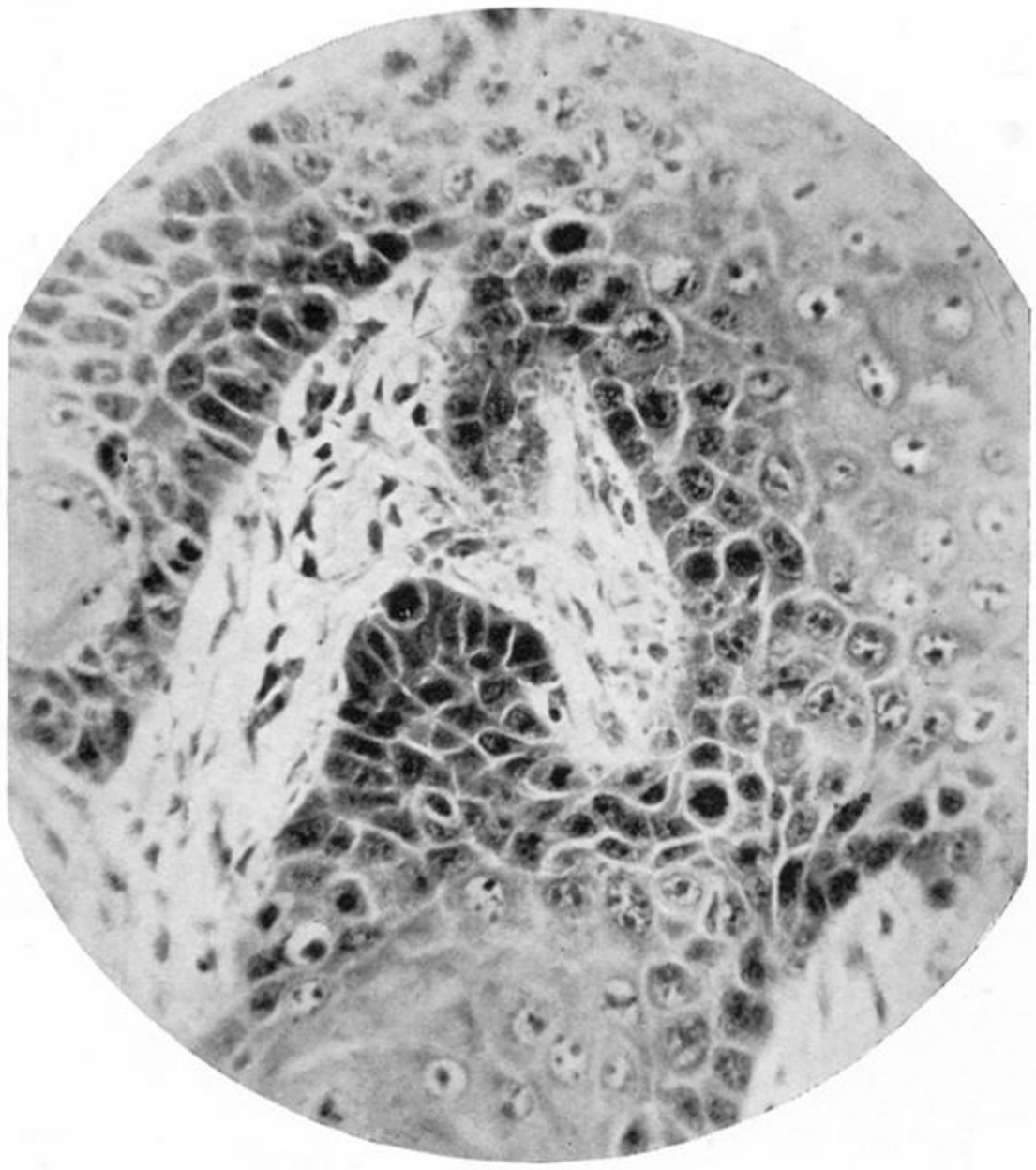

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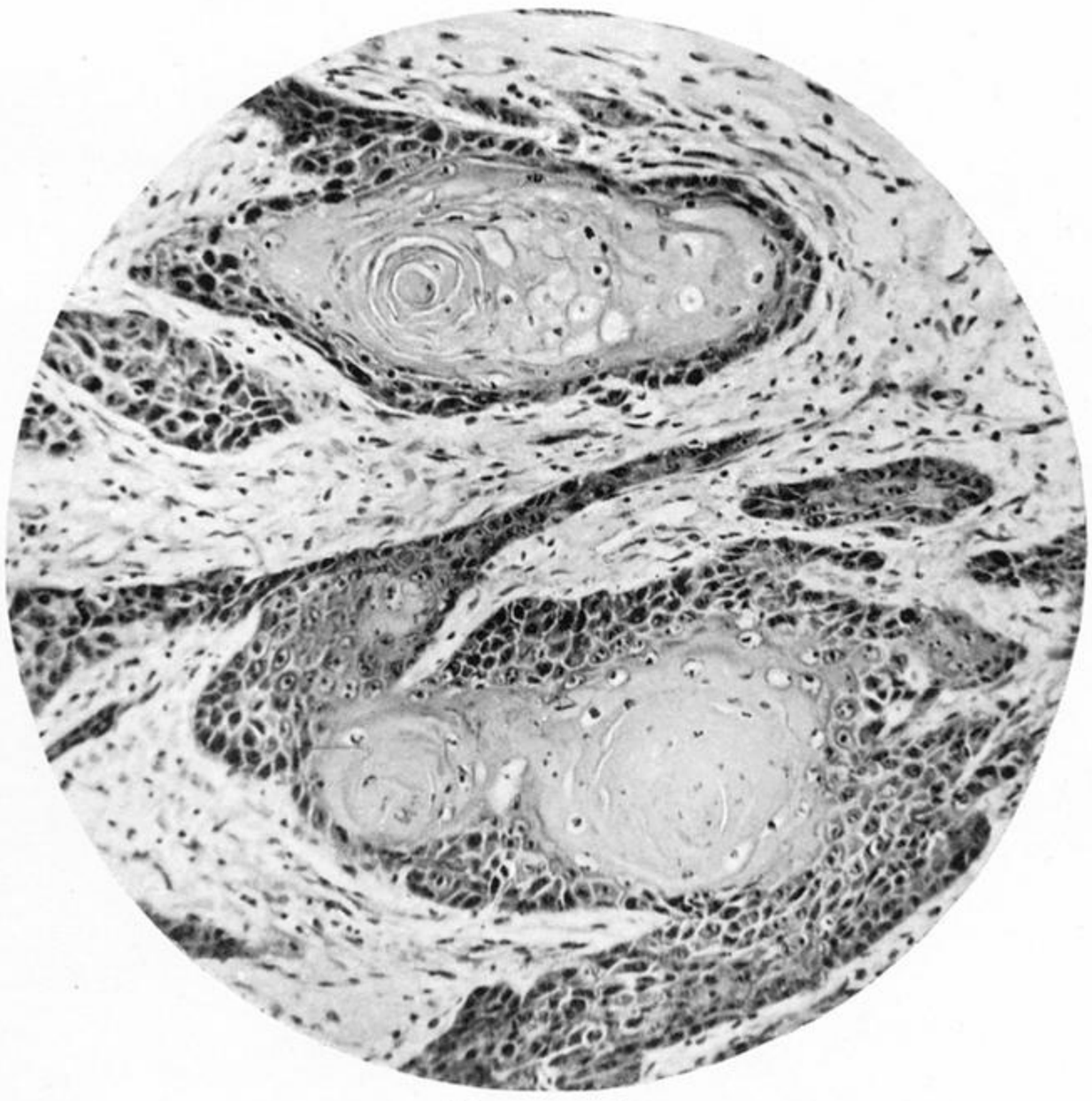

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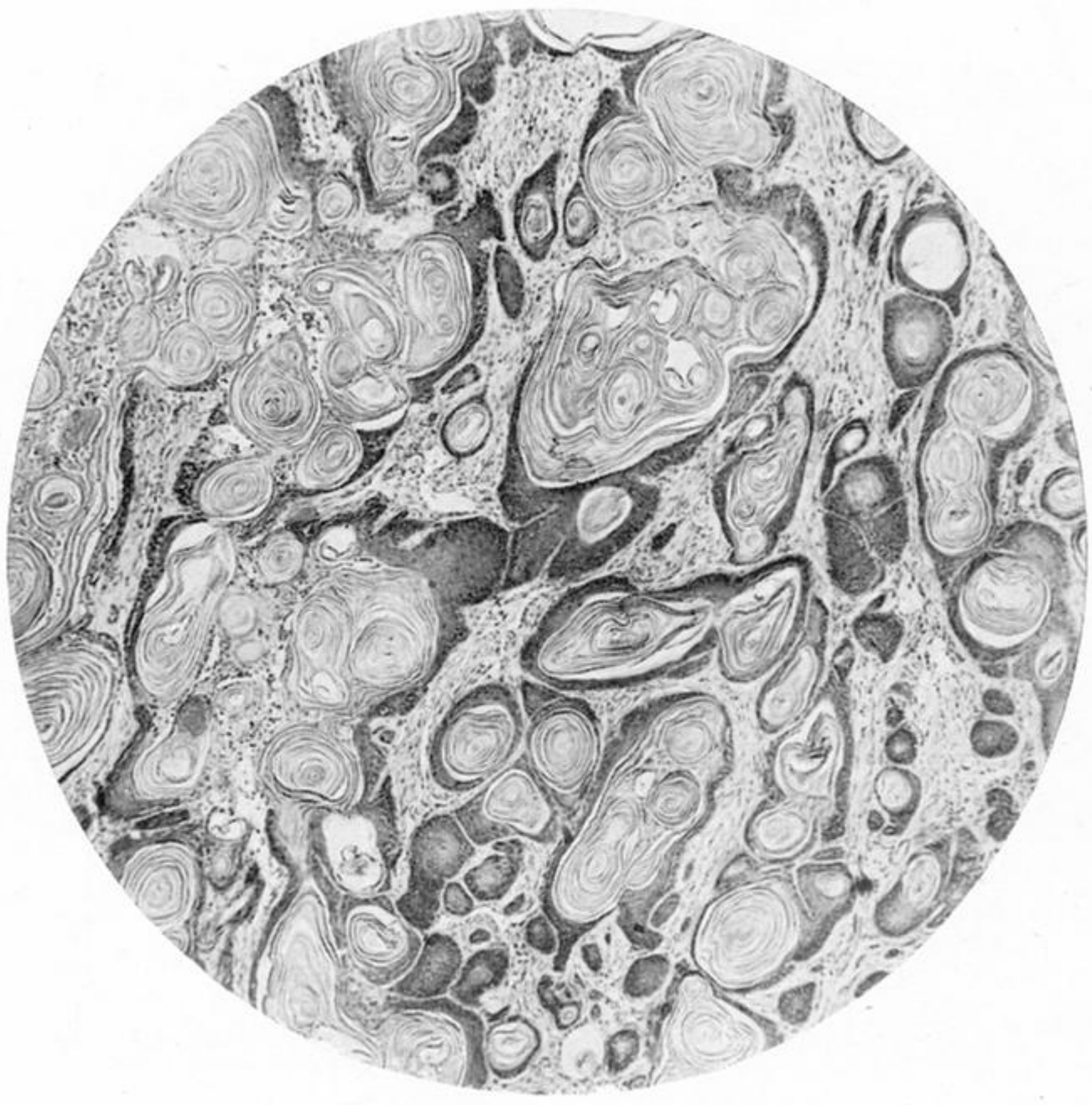

FIGURE 11

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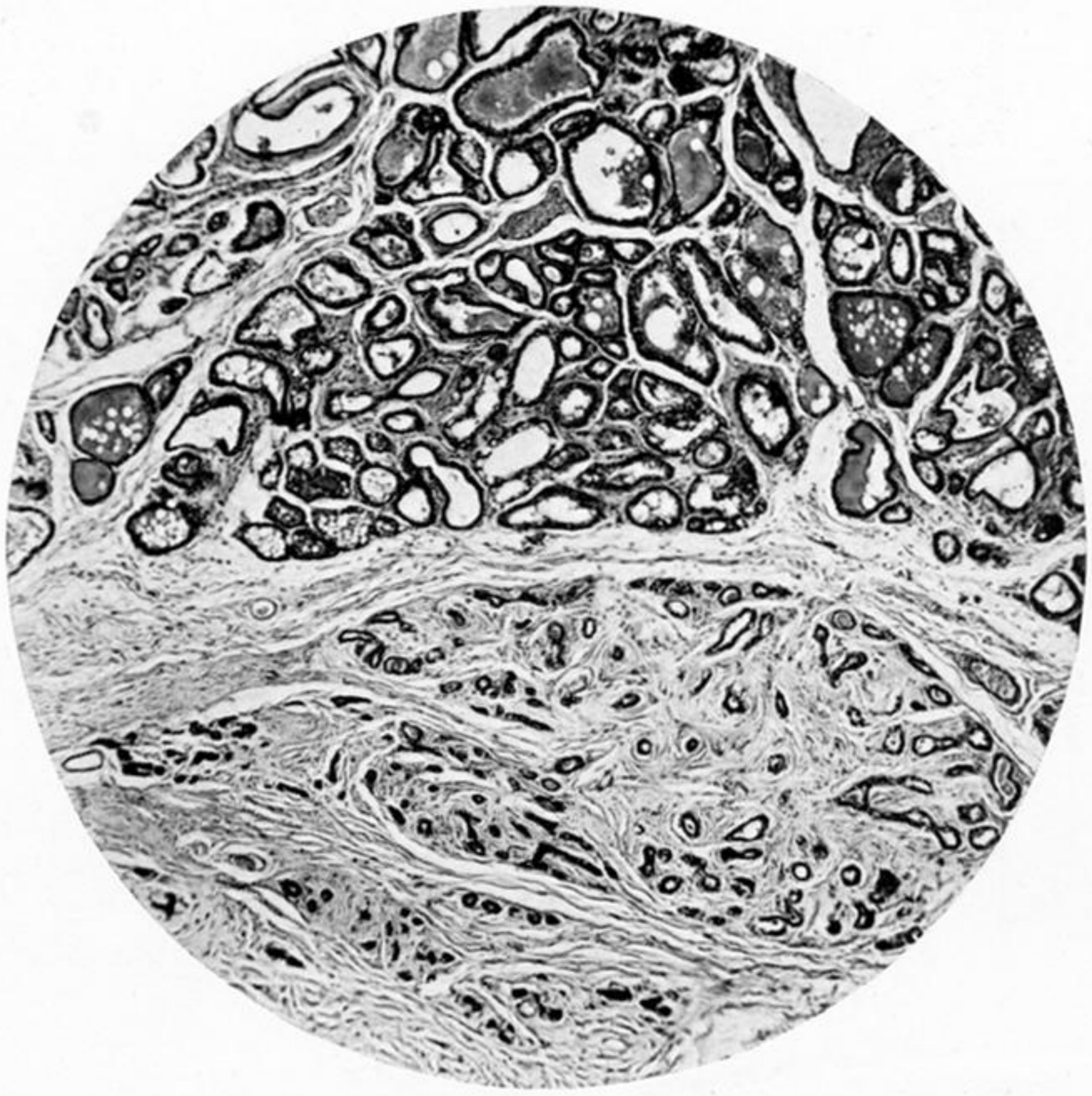

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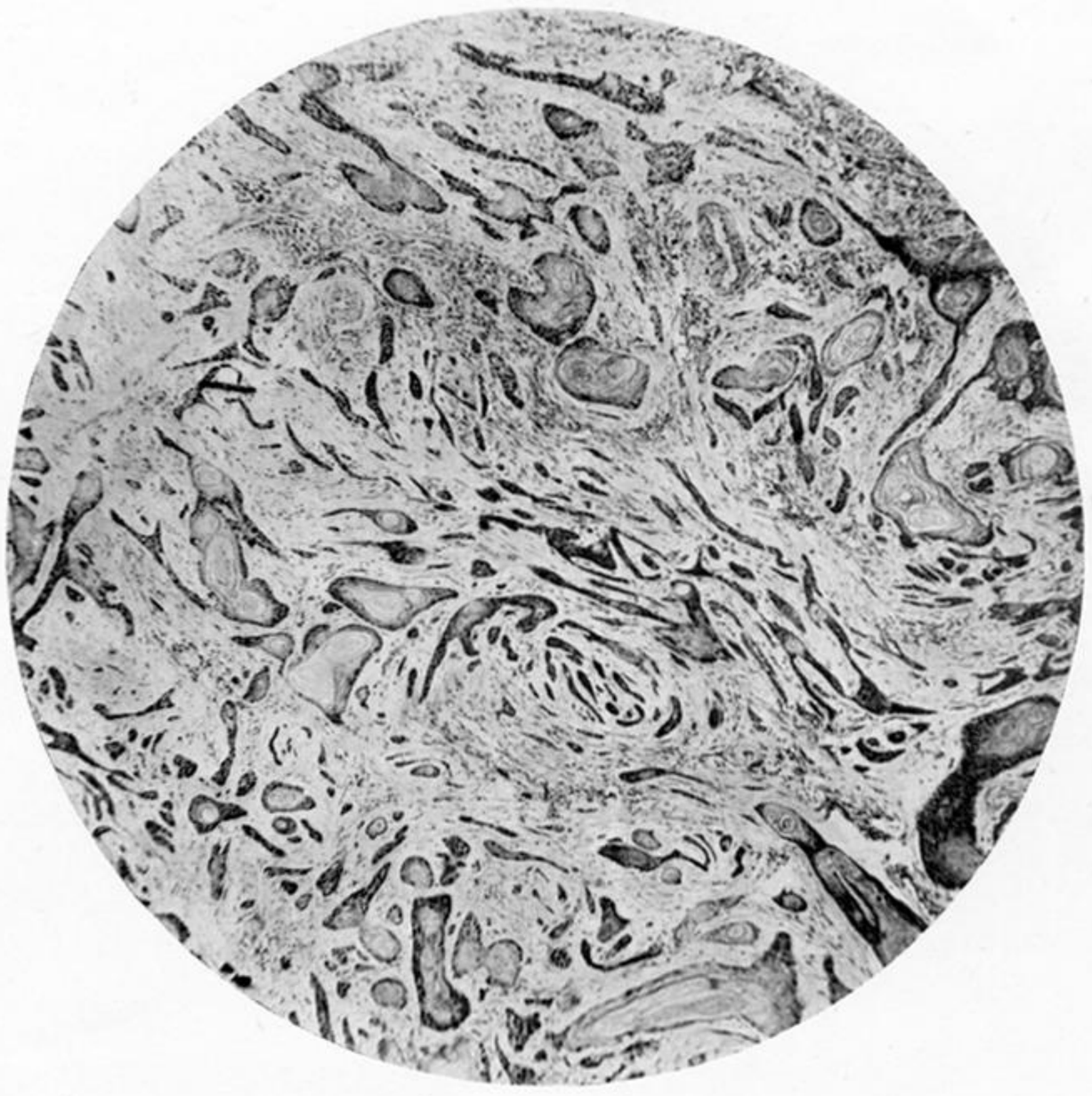
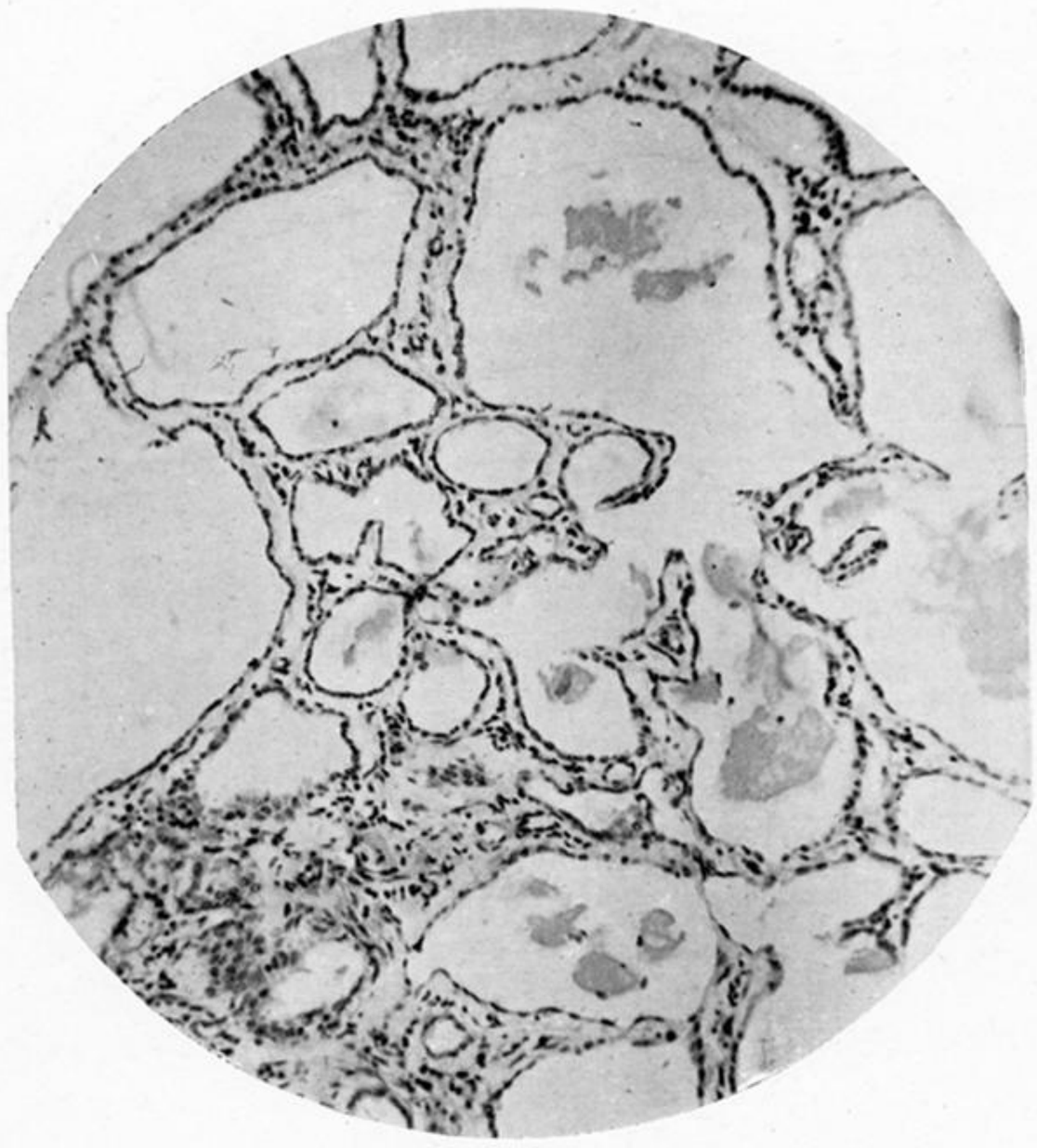


FIGURE 13



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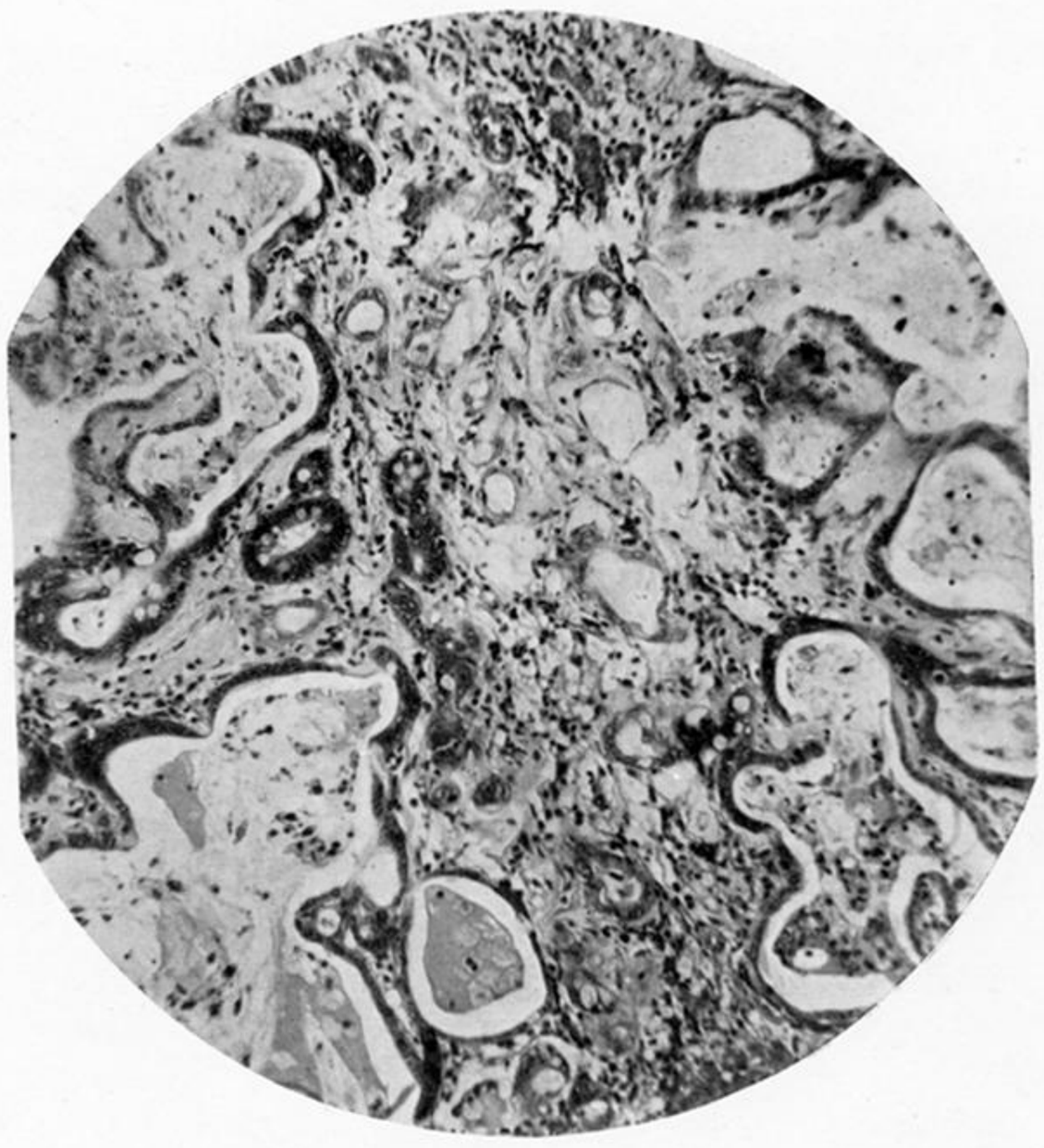

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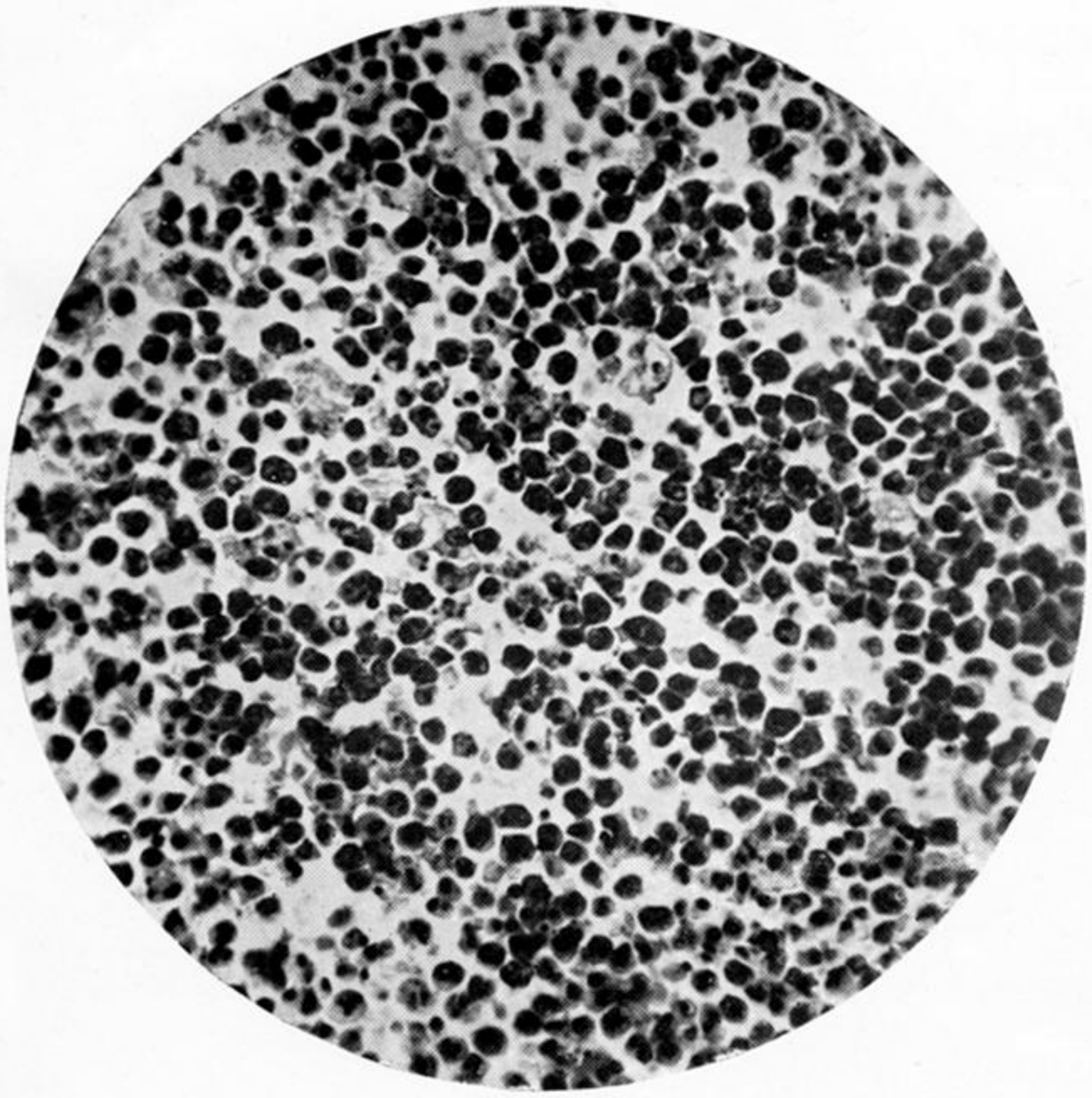
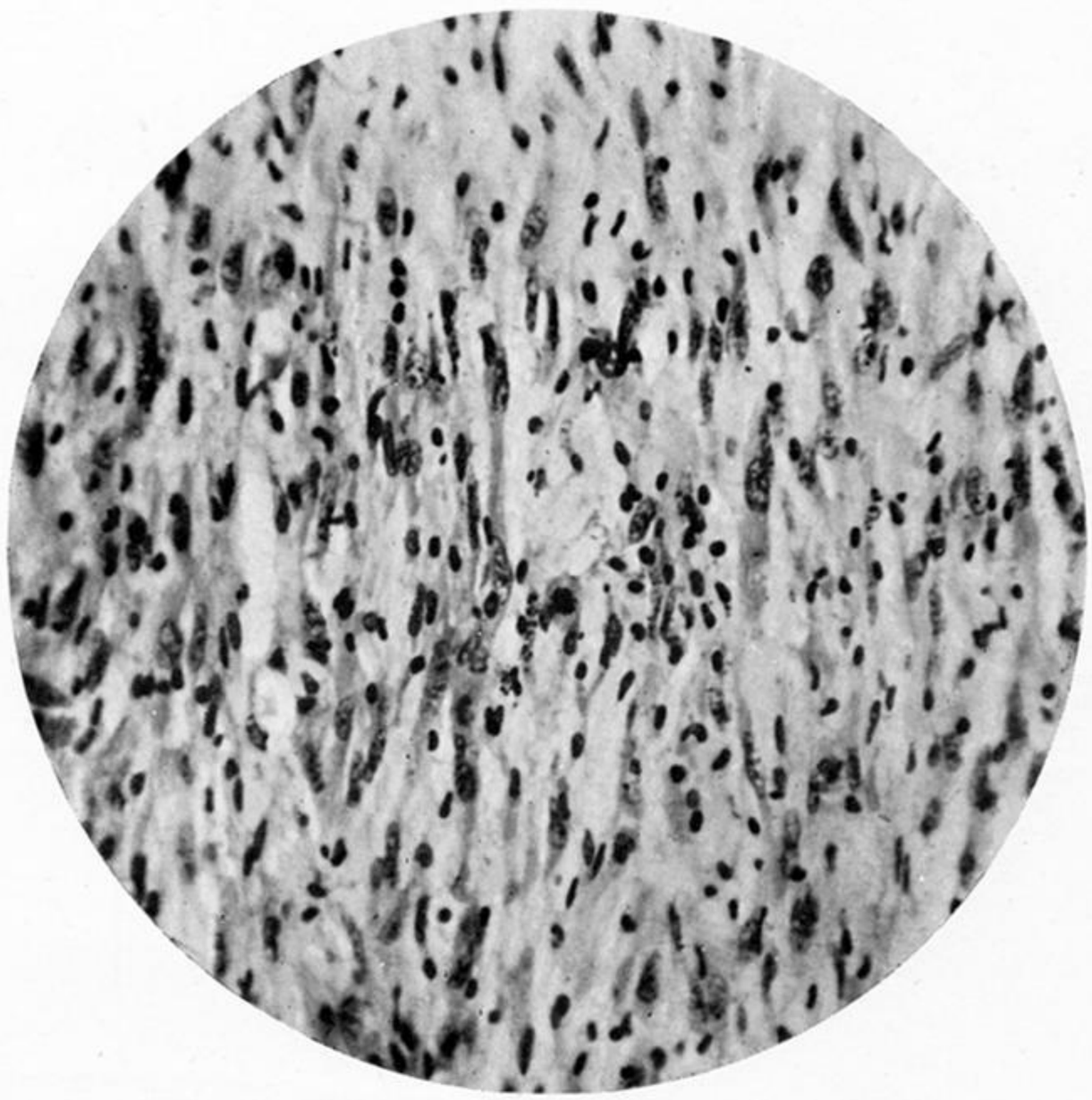


FIGURE 16



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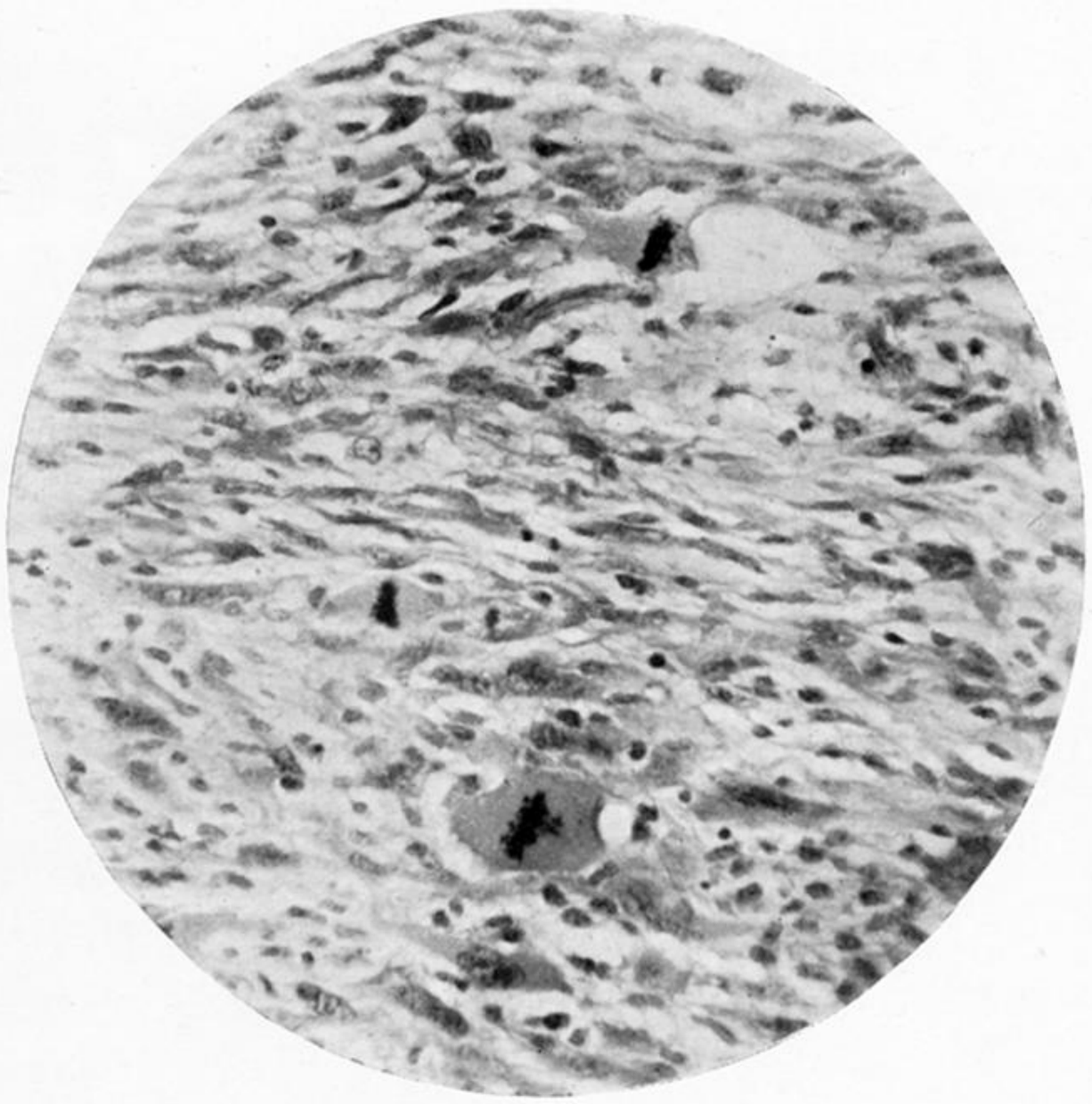

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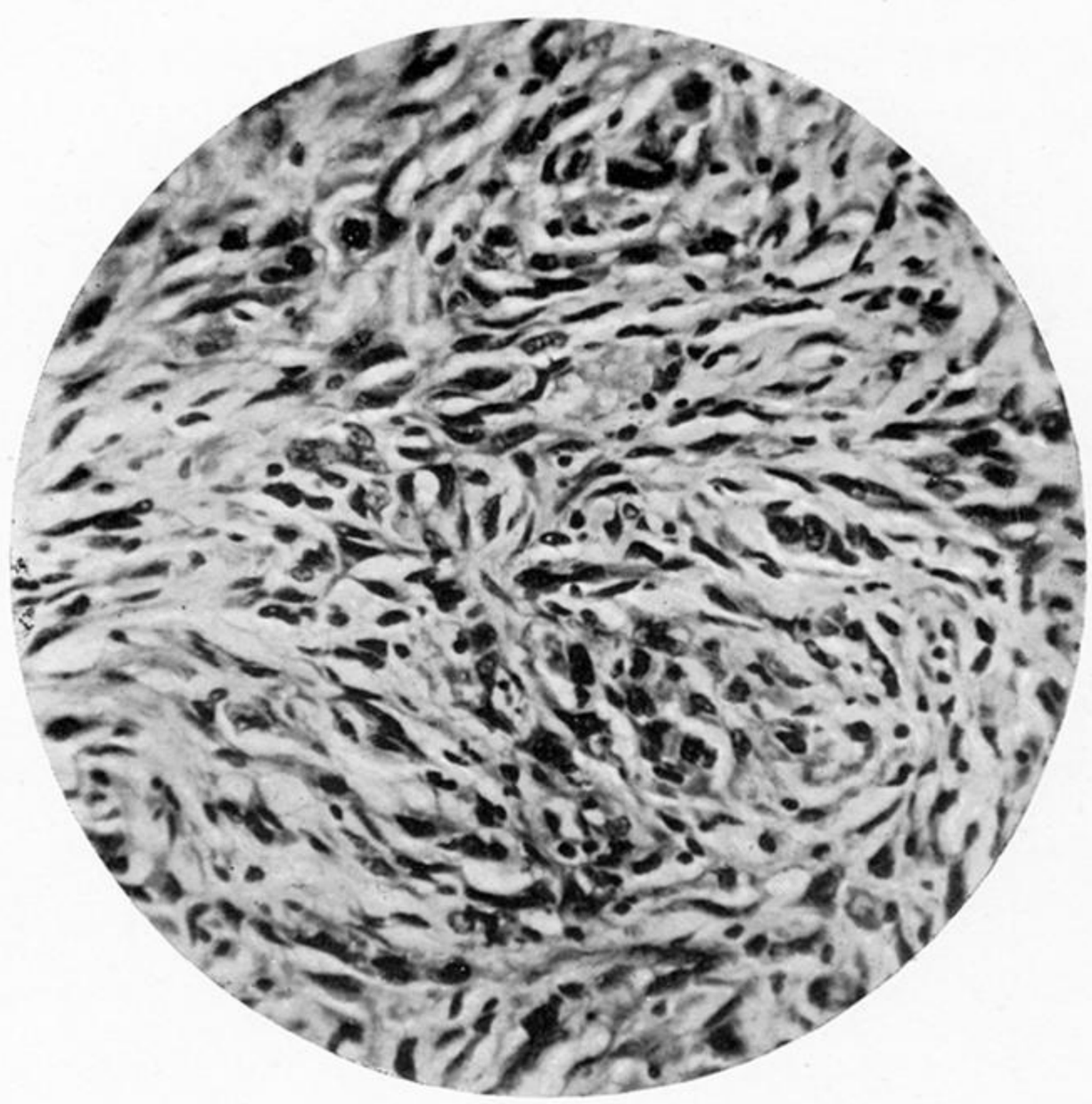

FIGURE 19