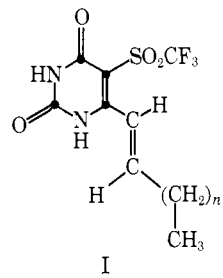
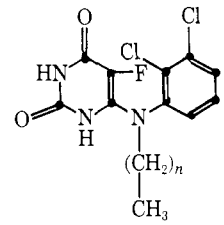
that a hydrophobic area exists beyond the N_1 position and at the 5 position, and that compounds with an acidic hydrogen at the 1 position make good inhibitors. Since the 6-anilinouracils show good inhibition, incorporation of this moiety would be advantageous. The following compounds would be predicted to be good inhibitors of thymidine phosphorylase. The long alkyl chain in each compound would be expected to bridge to the hydrophobic area beyond the N_1 position. The 5-SO_2CF_3 group would provide hydrophobic character as well as lowering the pK_a of the N_1 hydrogen. The dichlorophenyl moiety would provide binding in the area where the 6-anilinouracils bind. This study does



I



II

support in a quantitative way the qualitative findings of Baker and coworkers. It also offers ideas for the development of more effective inhibitors.

Mixed Bifunctionality. III. Antitumor Activity of Sesame Oil Solutions of Simple Alkylating Derivatives of Polynuclear Hydrocarbons[1]

RICHARD M. PECK AND ANNA P. O'CONNELL

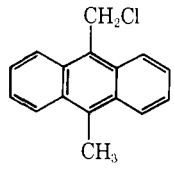
The Institute for Cancer Research, Philadelphia, Pennsylvania 19111

Received February 18, 1970

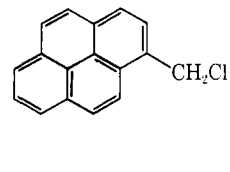
Antitumor activity of chloromethyl aromatic hydrocarbons is enhanced by administration in sesame oil solution compared with saline suspension. Microgram amounts of the most active compounds are curative in the Ehrlich mouse ascites tumor. Structural variation of the polycyclic aromatic radical has been related to antitumor activity. These relationships only partially correspond with those when mustard groups rather than the chloromethyl group furnish the alkylating function. The previously noted high activity of chloromethyl aromatic hydrocarbon *vs.* the mustard-resistant S-37 tumor has been studied in detail.

We have previously reported the discovery that antitumor activity is conferred on a monofunctional N mustard,[2,3] on S half mustard,[4] and on a simpler alkylating function[5] by the presence of a polynuclear moiety in the same molecule. Several simple chloromethyl aromatic hydrocarbons were among the most potent compounds. This discovery was surprising since these are hydrophobic, insoluble chemicals which were given as fine suspensions in saline to tumor-bearing mice.

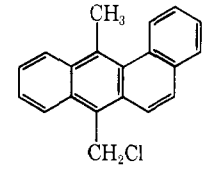
In an effort to determine whether greater *in situ* availability would affect the antitumor activity of such compounds as I–III, they were injected intraperitoneally as solutions in sesame oil into mice bearing ascites tumors.[2–5] This mode of administration in fact



I



II



III

markedly increased both the activity and the toxicity of I–III compared with these properties when I–III were given in suspension. In view of this enhancement of potency, further structural variation of the aromatic group was studied (see Table I and section on Biological Results). In addition, the previously noted efficacy of some of these compounds against the mustard-resistant S-37 tumor[5] has been examined (see Table II).

To obtain the previously unreported compounds in Table I, direct chloromethylation was not attempted, since it had been found that the impurity from even a small amount of excess chloromethylation can give a false enhancement of activity.[5] Where possible, the aldehyde was the preferred intermediate, followed by (1) reduction either with $LiBH_4$ or $NaBH_4$, and (2) action of dry HCl. Several of the required aldehydes are known, and formylation of 2,9-dimethylanthracene gave the 10-carboxyaldehyde. However, 1,9-dimethylanthracene gave an intractable tar. The only method found to obtain this and other hydroxymethylanthracenes bearing alkyl substituents in the outer rings was *via* the ICH_2 derivatives available from the anthraquinone.[6] Reaction of these iodo compounds with moist Ag_2O gave variable yields of the $HOCH_2$ compound. Dry HCl yielded the $ClCH_2$ compound in every case except the same 1,9-dimethyl derivative. In one case, **7** in Table I, PCl_3 in C_6H_6 was employed.[7] Table III lists the intermediate $HOCH_2$ compounds not previously reported.

Experimental Section

Melting points were taken in open capillary tubes in a Hershberg apparatus using total immersion thermometers and are reported as uncorrected values. Where analyses are indicated

(1) Supported by Research Grants CA-02975, CA-06927, and FR-05539 from the National Institutes of Health, U. S. Public Health Service and by an appropriation from the Commonwealth of Pennsylvania.

(2) R. M. Peck, R. K. Preston, and H. J. Creech, *J. Org. Chem.*, **26**, 3409 (1961).

(3) (a) R. K. Preston, R. M. Peck, E. R. Breuninger, A. J. Miller, and H. J. Creech, *J. Med. Chem.*, **7**, 471 (1964); (b) R. M. Peck, E. R. Breuninger, A. J. Miller, and H. J. Creech, *ibid.*, **7**, 480 (1964).

(4) (a) R. M. Peck, A. P. O'Connell, and H. J. Creech, *ibid.*, **9**, 217 (1966); (b) R. M. Peck, A. P. O'Connell, and H. J. Creech, *ibid.*, **10**, 37 (1967).

(5) R. M. Peck, A. P. O'Connell, and H. J. Creech, *ibid.*, **13**, 284 (1970).

(6) G. M. Badger and R. S. Pierce, *J. Chem. Soc.*, 2314 (1950).

(7) W. E. Bachmann and M. Carmack, *J. Amer. Chem. Soc.*, **63**, 2494 (1941).

Table I

Alkylating Agents *vs.* the Ehrlich Ascites Tumor

No.	Compd	Antitumor activity[a] Range, μmol/kg	Degree	Yield, %	Mp, °C	Formula[b]
	A. Monofunctional					
1	4-Chloromethyl-1-methylnaphthalene	40–150 (60–300)	2.2			$C_{12}H_{11}Cl$
2	9-Chloromethylphenanthrene[c]	175–250	2.1			$C_{15}H_{11}Cl$
3	10-Chloromethyl-9-methylphenanthrene	10–250 (35–300)	2.2			$C_{16}H_{13}Cl$
4	9-Chloromethylanthracene	0.5–15 (1–125)	2.2			$C_{15}H_{11}Cl$
5	10-Chloromethyl-9-methylanthracene	1.5–50 (4–100)	2.4			$C_{16}H_{13}Cl$
6	10-Chloromethyl-9-ethylanthracene	4–80	2.1	51	174–178 dec	$C_{17}H_{15}Cl$
7	10-Chloromethyl-2,9-dimethylanthracene	1.5–45	2.2	35	145–155	$C_{17}H_{15}Cl$
8	10-Chloromethyl-2,3,9-trimethylanthracene	4–200	2.2	47	141–143 dec	$C_{18}H_{17}Cl$
9	10-Chloromethyl-2,6,9-trimethylanthracene	2–80	2.2	31	>250	$C_{18}H_{17}Cl$
10	10-Chloromethyl-9-chloroanthracene[d]	0.1–6	2.3	79	172–174	$C_{15}H_{10}Cl_2$
11	7-Chloromethylbenz[*a*]anthracene	0.2–4 (0.8–20)	2.3			$C_{19}H_{13}Cl$
12	7-Chloromethyl-12-methylbenz[*a*]anthracene	0.2–10 (2–175)	2.4			$C_{20}H_{15}Cl$
13	1-Chloromethylpyrene	0.1–6 (0.8–35)	2.2			$C_{17}H_{11}Cl$
14	5-Chloromethylbenzo[*a*]pyrene	0.8–80	2.3	70	215–218 dec	$C_{21}H_{13}Cl$
15	7-Iodomethyl-12-methylbenz[*a*]anthracene	0.8–12 (20–350)	2.2			$C_{20}H_{15}I$
16	9-(2-Chloroethyl)anthracene[e]	Inactive				$C_{16}H_{13}Cl$
17	9-Chloromethyltriptycene[e]	Inactive				$C_{21}H_{15}Cl$
18	2-Chloromethylbenzimidazole[f]	200–250	1.9			$C_8H_7ClN_2$
19	9-Chlorofluorene[f]	Inactive				$C_{13}H_9Cl$
	B. Bifunctional					
1	1,4-Bis(chloromethyl)benzene	Inactive; toxic at 250				$C_8H_8Cl_2$
2	3,6-Bis(chloromethyl)durene	10–250 (50–180)	2.3			$C_{12}H_{16}Cl_2$
3	α,α',2,3,5,6-Hexachloro-*p*-xylene	Inactive; toxic at 125				$C_8H_4Cl_6$
4	9,10-Bis(chloromethyl)anthracene	0.1–4 (0.3–30)	2.4			$C_{16}H_{12}Cl_2$

[a] No compound was tested beyond 250 μmoles/kg. Values in parentheses are activity ranges previously found for the same compound given as a fine saline suspension. These data and sources of these compounds are given in the preceding paper.[5] [b] Previously unreported compounds, for which melting point and yield are tabulated, were analyzed for C, H, Cl. [c] Previously reported by J. W. Cook, A. Dansi, C. L. Hewett, J. Iball, W. V. Mayneord, and E. Roe, *J. Chem. Soc.*, 1319 (1935), mp 100–100.5° from chloromethylation of phenanthrene; our sample melted at 100–101°. [d] Identical with the previously reported *x*-chloro-9-chloromethylanthracene[4b] resulting from the action of excess $SOCl_2$ on either 9-hydroxymethylanthracene or 2-(9-anthrylmethylthio)ethanol. [e] Kindly supplied by Dr. E. C. Kornfeld, Eli Lilly & Company. [f] Commercially available.

Table II

Antitumor Activity *vs.* S-37 Mouse Ascites

Compd	Antitumor activity Range, μmoles/kg	Degree
9-Chloromethylanthracene	1–10	2.1
10-Chloromethyl-9-methylanthracene	4–40	2.1
7-Chloromethylbenz[*a*]anthracene	0.4–10	2.1
7-Chloromethyl-12-methylbenz[*a*]anthracene	1–12.5	2.2
1-Chloromethylpyrene	1–8	2.4
9,10-Bis(chloromethyl)anthracene	0.4–8	2.3

only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

2,9-Dimethyl-10-anthraldehyde.—A mixture of 7.2 g of 2,9-dimethylanthracene[8] and 6.3 ml each of $POCl_3$, methylformanilide, and *o*-$Cl_2C_6H_4$ was heated for 0.75 hr with swirling on the steam cone, cooled, and decomposed with aq NaOAc in the presence of a little petroleum ether (bp 30–60°). The filtered crude product (7.9 g) was crystallized from HOAc to give 4.85 g (59%), mp 95–98.5°. An analytical sample, mp 99–100.5°, was obtained by crystallization from C_6H_6–petroleum ether (bp 30–60°). *Anal.* ($C_{17}H_{14}O$) C, H, O.

2-[α-Hydroxy-α-(2-tolyl)ethyl]benzoic Acid Lactone.—To a solution of 4.8 g of 2-(*o*-toluyl)benzoic acid in 100 ml of C_6H_6 and 50 ml of Et_2O was added slowly with stirring 20 ml of 3 *M* MeMgBr in Et_2O. After overnight standing and decomposition with saturated NH_4Cl, the mixture was shaken with ice-cold dil HCl and washed. Extraction with portions of 1 *N* Na_2CO_3 gave a solution which on acidification gave an oil which slowly crystallized and was then no longer soluble in cold alkali. Recrystallization from C_6H_6–petroleum ether gave 2.15 g of the lactone (45%), mp 100–100.5°. *Anal.* ($C_{16}H_{14}O_2$) C, H; C: calcd, 80.64; found, 81.17, 81.16.

2-[α-(2-Tolyl)ethyl]benzoic Acid.—A mixture of 7.25 g of the lactone, 35 ml of 50% NaOH, and 350 ml of EtOH was refluxed 18 hr, concentrated by one-half, and 150 ml of H_2O and 20 g of Zn dust were added, followed by 15 ml of 50% NaOH and 3-hr reflux with stirring while 100 ml was distilled. After another addition of 20 g of Zn dust and 20 ml of 50% NaOH, reflux with stirring was continued 20 hr and the hot solution was filtered and the residue washed with hot H_2O. The filtrate was made strongly acidic. The product was collected and reprecipitated from dilute K_2CO_3 to give 7.3 g (nearly quantitative), mp 153–155°. A sample recrystallized from C_6H_6–petroleum ether melted at 155–156°. *Anal.* ($C_{16}H_{16}O_2$) C, H; C: calcd, 79.98; found, 79.38.

4,10-Dimethylanthrone.—To 55 ml of anhydrous HF was added 6.5 g of 2-[α-(2-tolyl)ethyl]benzoic acid and the mixture swirled occasionally over a 20-min period and poured on ice. The gum, which formed slowly, crystallized on rubbing, was collected, and washed. Crystallization from MeOH gave 4.6 g (77%), mp 85–86°. *Anal.* ($C_{16}H_{14}O$) C, H, O.

1,9-Dimethylanthracene.—To a stirred solution of 2 g of $LiBH_4$ in 250 ml of dry Et_2O was added a solution of 4.5 g of dimethylanthrone in 20 ml of warm C_6H_6. After overnight stirring, the mixture was decomposed with dilute HOAc, separated, and the organic layer washed, dried, and concentrated. Dilution with EtOH gave 3.8 g, mp 92–94°. Recrystallization from hexane gave 3.25 g (78%), mp 94–95°. *Anal.* ($C_{16}H_{14}$) C, H. Attempted formylation by the method used for 2,9-dimethylanthracene gave an intractable mass, probably due to *para* substitution in the terminal ring.

2,9-Dimethyl-10-hydroxymethylanthracene (4, Table III). Method A.—To a stirred solution of 1.0 g of $LiBH_4$ in 250 ml of dry Et_2O was added a solution of 4.7 g of 2,9-dimethyl-10-an-

(8) E. deB. Barnett and N. F. Goodway, *J. Chem. Soc.*, 1758 (1929).

TABLE III
HYDROXYMETHYL HYDROCARBON DERIVATIVES

No.	Compd	Method[a]	Yield, %	Mp, °C	Formula[b]
1	9-Chloro-10-hydroxymethylanthracene	A	41	226–228 dec	$C_{15}H_{11}ClO$
2	9-Ethyl-10-hydroxymethylanthracene	A	31	180–183 dec	$C_{17}H_{16}O$
3	1,9-Dimethyl-10-hydroxyethylanthracene	B	15	139–149	$C_{15}H_{16}O$
4	2,9-Dimethyl-10-hydroxyethylanthracene	A	78	180–181	$C_{17}H_{16}O$
5	2,3,9-Trimethyl-10-hydroxyethylanthracene	B	51	148–152	$C_{18}H_{18}O$
6	2,6,9-Trimethyl-10-hydroxyethylanthracene	B	15.5	>270	$C_{18}H_{18}O$
7	5-Hydroxymethylbenzo[*a*]pyrene[c]	A	82	229–230.5	$C_{21}H_{14}O$

[a] See Experimental Section. Yields for method B are calculated from the crude iodomethyl compound; these were consistently prepared in 70–90% yields from the anthraquinones. [b] All compounds were analyzed for C, H. [c] Benzo[*a*]pyrene-5-carboxaldehyde prepared as by L. F. Fieser and E. B. Hershberg, *J. Amer. Chem. Soc.*, **60**, 2542 (1938).

thraldehyde in a small volume of warm C_6H_6. The mixture was stirred 1 hr, decomposed with ice and HOAc, and filtered, giving 3.1 g of crude product, mp 181–183°. An additional 1.2 g was obtained by concentration of the organic layer, mp 177–179°. The combined product was recrystallized from EtOH to give 3.7 g (78%) of **4** (Table III).

10-Hydroxymethyl-2,3,9-trimethylanthracene (5, Table III). Method B.—A mixture of 7.9 g of 10-iodomethyl-2,3,9-trimethylanthracene (crude; prepared by the procedure of Badger and Pierce[6]) and 400 ml of dioxane was stirred and heated. When the solution became clear, the moist, washed Ag_2O precipitated from 4.2 g of $AgNO_3$ was added suspended in 80 ml of H_2O. After rapid heating to reflux, vigorous stirring was continued 0.5 hr folllwed by hot filtration and washing. The filtrate was taken to dryness *in vacuo* and the residue leached twice with hot alkali-distilled EtOH, leaving 0.5 g of insoluble material. The EtOH solution was diluted with H_2O and cooled; the filtered product was recrystallized from C_6H_6–petroleum ether to give 2.8 g, mp 150–155°, on rapid heating. The analytical sample from a previous preparation had the melting point shown in Table III.

10-Chloromethyl-9-ethylanthracene.—Dry HCl was rapidly bubbled through a solution of 1.0 g of 9-ethyl-10-hydroxymethylanthracene in 50 ml of EtOH and 20 ml of C_6H_6 for 15 min; during this period the product crystallized. Cooling and filtration gave 0.7 g; recrystallization from C_6H_6–petroleum ether gave 0.55 g, mp 174–178° dec. Many other chloromethyl products did not separate from the reaction mixture; in these cases solvent was removed *in vacuo* and the product crystallized from C_6H_6–petroleum ether.

Biological Results and Discussion

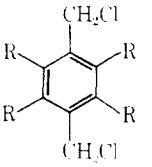
In our usage of the Ehrlich ascites tumor in this laboratory for many years, control animals injected with saline alone on days 1, 2, and 3 following an intraperitoneal implant of 7×10^6 ascites cells have shown a consistent mean survival time of 15–17 days.[2–5] When both control and experimental groups were injected with sesame oil, in which the halomethyl hydrocarbons are soluble, it was noted that the control group's mean survival time was shortened. Since the shortening is in proportion to the amount of oil injected, it appears that sesame oil alters the properties of ascitic fluid in a manner favorable to the growth of tumor cells. This new variable was standardized by giving injections of compounds in 0.3 ml of sesame oil, the same volume given the controls; under these conditions mean survival time of controls was shortened by 5 days, *i.e.*, to 10–12 days. Activity is then reckoned on the same basis as the previous work: the activity range begins at the lowest dosage (expressed in μmoles/kg) which produces an 80% increase in mean survival time over controls—a degree value of 1.8, and ends with a dosage where partial toxicity reduces mean survival again to 1.8 after the optimum range has been passed. At intermediate dosages the arbitrary maximum of 3.0 (200% increase in mean survival) is frequently reached. Experiments are terminated at this point (30–36 days) to conserve animal colony space and because experience has shown that animals surviving to this point seldom show recurrence of tumor when held for longer periods. Since the "degree figure" given in Tables I and II is an average including the two limiting figures of 1.8 as well as 3–8 intermediate dosages, a degree figure of 2.2–2.4 denotes a highly active compound; a favorable ratio of high:low dosage range is a second desirable property. Of unique interest is the potency; no compound of any kind previously tested in this system has been effective at a dose of 0.1 μmole/kg (HN2 itself, a bis N mustard, has an active range of 3–8).

Structure–activity comparisons from Table I show broad agreement with the generalizations drawn from mustard derivatives of aromatic hydrocarbons,[4,5] but with definite divergences. Progression from the simple naphthalene derivative to the phenanthrenes and anthracenes shows a great increase in potency, paralleling studies of carcinogenicity, and this continues, though at a lesser rate, into the benzanthracene derivatives. However, addition of a *meso* Me, a powerful enhancer of potency in the mustard derivtives, gives compounds of somewhat *reduced* potency (**5** *vs.* **4**, and **12** *vs.* **11**), though the degree averages are somewhat better. Substitution of Et at the *meso* position (**6**) again[5] gives a relatively unfavorable result. A *meso* Me in the phenanthrene series (**3** *vs.* **2**) is very favorable, but neither of these compounds is in the highly potent range. Substitution of Me groups in the outer rings (**7** through **9**) leads to loss of potency; this parallels one such substitution among the mustard derivatives[5] and subsequent data (unpublished) on two other analogs. The favorable effect of the benz- group (**11** *vs.* **4** and **12** *vs.* **5**) is contradicted in the pyrene series (**14** *vs.* **13**); however, the additional ring added is not strictly analogous. The very favorable effect of a *meso*-Cl atom is to be pointed out (**10**).

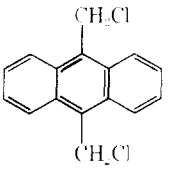
Two basic requisites for activity appear to be (1) an aromatic moiety which approaches planarity and for which a moderate size and complexity seem essential, and (2) a highly reactive alkylating function. That an optimum range of chemical reactivity is essential for 2 is shown by comparisons of **12** *vs.* **15**, and **4** *vs.* **16**. Compound **15** is less active biologically than **12** in spite of much higher chemical activity; conversely **16**, with the reactivity of a typical alkyl halide rather than the more reactive arylmethyl halide (**4**), loses all biological activity. Two related nonalkylating compounds not

included in the table are 9,10-dimethylanthracene and 10-hydroxymethyl-9-methylanthracene. They are inactive. The "right" kind of chemical reactivity is possessed by **18** and **19**, but apparently the aromatic character is insufficient for more than minimal activity. Compound **17** lacks both qualifications. In view of the profound variation seen in Table I, other structural and substitutional investigations seem worthwhile. These findings appear to us to support further the mechanism of activity proposed in the preceding paper,[5] namely that these chemicals serve as DNA cross-linking agents whereby intercalation of the aromatic portion of the molecule is followed by chemical reaction between the alkylating group and one of the nucleophiles present in the DNA helix.

In the bifunctional series (Table IB) the comparison between **1** and **2** reinforces in a clear-cut manner the paramount contributions of geometry to activity of a molecule, a point emphasized in the preceding paper.[5] The enhancement of activity by administration in sesame oil reveals moderate activity in a simple benzene derivative (IV, R = Me), where none at all exists in the absence of the four Me groups (IV, R = H). Our view is that this activity derives from the ability of these groups partially to mimic the two outer rings of the very active anthracene (V; **4** in Table IB). The activity of V is such that the total dosage at the lower end of its active range is approximately 2 μg. divided among 3 doses.



IV



V

Table II shows corresponding figures for some of the most active compounds against the S-37 mouse ascites tumor, which is not cured by any mustard compound, including those in clinical use. In a few isolated instances, 50–60% increases in survival were formerly obtained—a degree value of 1.5–1.6. Table II generally, but not uniformly, presents a parallel to the figures in Table I showing a moderately less curable tumor with the curative range shifted upward at both ends—the compounds are appreciably less potent but usually slightly less toxic compared to their use against the Ehrlich tumor. The same protocol of 0.3 ml of sesame oil in both controls and experimentals was used with this tumor, although sesame oil had no appreciable effect on the controls' mean survival time, in contrast to its effect on the Ehrlich tumor-bearing mice.

Acknowledgment.—The authors are indebted to Dr. Jenny Pickworth Glusker and to Dr. Hugh J. Creech for helpful discussion. Assistance by Mrs. Gladys Bates and Mr. Joseph Kolb is also acknowledged.

Electron-Donating Properties of Phenothiazine and Related Compounds

John E. Bloor,

Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37916

B. R. Gilson, R. J. Haas,

Chemistry Department, University of Virginia, Charlottesville, Virginia 22901

and Charles L. Zirkle

Smith, Kline, and French Laboratories, Philadelphia, Pennsylvania

Received February 20, 1970

The relative electron-donating properties of phenothiazine and related compounds containing C, N, O, and S atoms have been determined by measuring the maximum of the charge transfer band on complexation with tetracyanobenzene. Phenothiazine does not display exceptional donor ability and also the N and not S is largely responsible for the observed moderate electron-donating properties. It is suggested that good tranquilizing activity is more likely to be conferred by flexibility of the active molecules or by the ability of S to form complexes with the localized electrons rather than by the electron-donating properties of the molecule as a whole. Self-consistent field Pariser–Parr–Pople molecular orbital calculations are also carried out for many of the molecules and used to interpret the observed uv spectra and ionization potentials.

The idea that the S atom in phenothiazine derivatives confers upon them exceptional electron-donating power,[1] has been very widely accepted[2] as has also the concept that the psychopharmacological behavior of certain phenothiazines is related to this remarkable electron-donating power.[2,3] In view of this wide acceptance and because the original ideas were founded on some very approximate Hückel molecular orbital calculations[1,4] it was thought worth while to carry out a systematic experimental determination of the relative electron-donating properties of a number of phenothiazines and related substances in order to either confirm or to disprove the original contention that the phenothiazines are indeed exceptional electron donors. Previous studies of the electron-donating properties of phenothiazines have been confined to solid state studies,[5] which are difficult to interpret in terms of the

(1) G. Karreman, I. Isenberg, and A. Szent-Gyorgyi, *Science*, **130**, 1191 (1959).

(2) C. Bodea and I. Silberg, *Advan. Heterocycl. Chem.*, **9**, 321 (1968).

(3) J. P. Mahieu, *Mol. Basis Some Aspects Mod. Actic. Proc. NATO Advan. Study Inst.*, **1965**, 83 (1965).

(4) (a) B. and A. Pullman, *Biochim. Biophys. Acta*, **35**, 535 (1959); (b) M. K. Orloff and D. D. Fitz, *ibid.*, **47**, 596 (1961).

(5) J. E. Lyons and J. C. Mackie, *Nature (London)*, **197**, 589 (1963).