of dihydrochloride (0.8 g, 60%) were recrystallized from absolute EtOH: mp 192–193°; nmr (DDS as standard in D_2O), δ 1.57 (2 H, multiplet, geminal cyclopropane *H*), 2.6 and 3.08 (1 H, multiplet, cyclopropane *H*), 7.34 and 8.63 (1 H, singlet, imidazole *H*). *Anal.* ($C_8H_{11}Cl_2N_3$) C, H, N.

5-(1-Triphenylmethyl-4-imidazolyl)-1,3-dioxo-1-methyl-2,3,-5,6-tetrahydrothiazinonium 2-Ylide (6).—When the mixture from the reaction of **2** with $Me_2S(O)(=CH_2)$ was poured into cold H_2O, instead of 25 m*M* HCl (*vide supra*), a colorless solid precipitated. Any cyclopropane compound (**3**) was extracted from the solid with several portions of Et_2O, and the remaining solid was filtered off and recrystallized (absolute EtOH), sintering at 134–138°, mp 234–235° dec. The sintering appeared to be due to crystallization with 1 mole of EtOH that was lost at 140–144° as shown in the nmr spectrum. The product was soluble in dilute acids. *Anal.* ($C_{28}H_{26}N_2SO_2 \cdot C_2H_5OH$) C, H, N. Spectra were recorded for the dried (EtOH-free) material: nmr δ 2.55 (2 H, doublet, J = 6.5 cps, CH_2CO), 3.33 (3 H, singlet, CH_3), 3.5–4.28 (3 H, multiplet, CH_2S superimposed on *CH*), 4.47 (1 H, singlet *CH*), 6.70 (1 H, singlet, imidazole *H*), 7.3 (16 H, multiplet, aromatic *H*, superimposed on imidazole *H*); mass spectrum, the product decomposed thermally, giving M^+ 390, probably losing CH_3SOH; fragmentation pattern as expected.

When **6** was dissolved in 0.05 *M* HCl and the solution was neutralized (NaOH) after 30 min, a colorless solid (**7**) precipitated out. Filtration and repeated recrystallization from MeOH gave a sample of mp 176–178°; M^+ 394; ir (cm^{-1}) 1775 (C=O), 1168 (C–O), and typical peaks for $C(C_6H_5)_3$; nmr, δ 2.75 (2 H, doublet, J = 7 cps), 3.7 (1 H, multiplet), 4.42 (2 H, multiplet), 6.7 (1 H, singlet, imidazole *H*), 7.28 (16 H, multiplet, aromatic *H* superimposed on imidazole *H*). *Anal.* Calcd for $C_{26}H_{22}N_2O_2$: C, 79.2; H, 5.58; N, 7.10. Found: C, 79.34; H, 5.58; N, 7.11.

A Novel Type of Substituted Piperazine with High Antiserotonin Potency[1]

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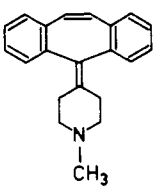
Speculation as to the structural relationship between phenbenzamine and cyproheptadine led to the synthesis of a series of tetracyclic compounds containing as a characteristic moiety a condensed piperazine ring resulting from the fixation of the ethylenediamine chain of phenbenzamine, whereas the two benzene nuclei of the latter are linked by a bond or a bridge of one or two carbon atoms. The piperazine ring system was formed by condensation of the respective diamines with diethyl oxalate (Riebsomer reaction), followed by reduction with diborane or $LiAlH_4$. These compounds (**4–7**) as well as the diphenylpiperazine (**3**) were tested pharmacologically and one of them, 2-methyl-1,2,3,4,10,14b-hexahydro-2H-pyrazino[1,2-*f*]morphanthridine (**5**), mianserin, proved to have an antiserotonin potency of the same order as cyproheptadine (**1**). In animals **5** was found to have a less pronounced CNS depressant effect and lower acute toxicity than **1**.

It is a common view that a pharmacological requirement of an antiallergic compound should be a high antihistamine activity. On the other hand it is believed that histamine is responsible for only some manifestations of anaphylactic reactions.[3] Indeed, during hypersensitivity reactions along with histamine other substances are released, serotonin being one of them.[4] In man, however, the role of serotonin as an allergic mediator is not likely,[5] although antiserotonin compounds proved to have clinically useful effects in disorders including vascular headaches and dumping syndrome.[6]

The object of the present work was to develop a compound with high antiserotonin potency. Of the many drugs capable of antagonizing one or more of the effects of serotonin, cyproheptadine (**1**) is of particular interest because its antagonism toward both histamine and serotonin is of a high order. The so called antihistaminics, as, for example, phenbenzamine (**2**), are much less potent, and in particular their antiserotonin activity is of a low order. Comparison of the structure of



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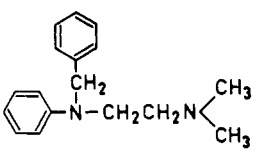

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these two compounds led to the question whether a structural modification of the phenbenzamine molecule might enhance its antiserotonin activity.

The most characteristic feature of the cyproheptadine molecule is the rigidity of its tricyclic ring system, which is connected with the N-containing fourth ring by a double bond, which again does not allow a free rotation. In the phenbenzamine molecule rotation of all groups is possible. Some structural similarities are, however, also present. Two benzene nuclei and one aliphatic tertiary N are present in both compounds. The second N of phenbenzamine is absent in cyproheptadine, but the double bond with its high electron density might play a comparable role with respect to its pharmacological activity. These considerations led to the idea of modifying the phenbenzamine molecule in a way that would result in similar structural rigidity. This may be done by attaching the ethylenediamine chain to the benzyl CH_2 and by connecting the benzene nuclei or introducing a bridge of one or two carbon atoms between them (compounds **4–7**).

(1) Presented in part at the IIIième Rencontre International de Chimie Thérapeutique, Paris, 1967.

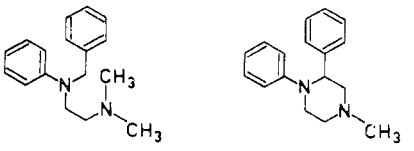
(2) Author to whom inquiries should be addressed.

(3) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," 3rd Ed, The Macmillan Co., New York, N. Y., 1965, p 622.

(4) J. H. Humphrey and R. Jaques, *J. Physiol.* (London), **128**, 9 (1955); *cf.* ref 3, p 621.

(5) Reference 3, p 653.

(6) (a) F. Sicuteri, *Intern. Arch. Allergy Appl. Immunol.*, **15**, 300 (1959); (b) L. P. Johnson, R. D. Sloop, and J. E. Jesseph, *J. Amer. Med. Ass.*, **180**, 493 (1962); *cf.* ref 3, p 652–653.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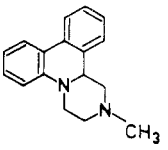
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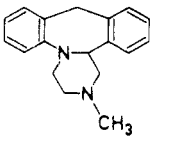
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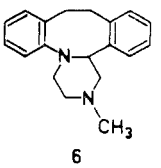
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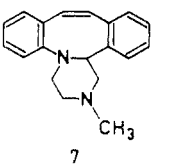
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Chemistry.—Synthetic routes were chosen in which the piperazine ring was constructed in the final steps (Scheme I). The substituted ethylenediamines (**10**, **14**, **20**, **28**) necessary for this purpose were prepared utilizing classical procedures. The Riebsomer condensation[7] of the diamines, thus obtained, with diethyl oxalate proved to be applicable for the formation of the four 2,3-diketopiperazines (**11**, **15**, **21**, **29**). The reduction of the diketo compounds to the substituted piperazines was performed using B_2H_6 in THF resulting in the "ring-closed phenbenzamines" (**3**–**6**). Compound **7**, in which a double bond is present between the benzene nuclei, was prepared by LAH reduction of the diketo compound **29**.

Pharmacology.—Pharmacological testing for antiserotonin potency included *in vitro* assays on the uterus of the rat and *in vivo* estimations on the rat paw edema and the guinea pig bronchoconstriction induced by serotonin (Table I). It appears from the low potency

TABLE I
ANTISEROTONIN POTENCY

Compound	Bridge	Rel potency: Rat uterus	Rel potency: Rat paw edema	Rel potency: Broncho-constr
3	Absent	0.05	2.4	1.5
4	Single bond	1	1.3	2.1
5 (mianserin)	$-CH_2-$	100	450	62
6	$-CH_2CH_2-$	0.2	2.4	2.1
7	$-CH{=}CH-$	1.3	2.4	5.8
Phenbenzamine		1	1	1
Cyproheptadine		50	510	75

of **3** that the fixation of the chain of phenbenzamine did not result in an appreciable increase of the desired antiserotonin activity. Nor was this achieved by introduction of a bond between the benzene nuclei, as shown by the low activity of **4**. Antiserotonin activity was, however, very much increased in **5** in which the phenyl groups are connected by a one-C bridge, whereas introduction of the 2-C bridges at the same point largely abolished the activity, as is observed in **6** and **7**.

Compound **5** (mianserin) proved to have, along with its high antiserotonin activity, which is of the same order as the activity of cyproheptadine, a less pronounced sedative effect and a lower acute toxicity as shown in pilot experiments on animals. These results will be published in more detail in the future.

(7) J. L. Riebsomer, *J. Org. Chem.*, **15**, 68 (1950).

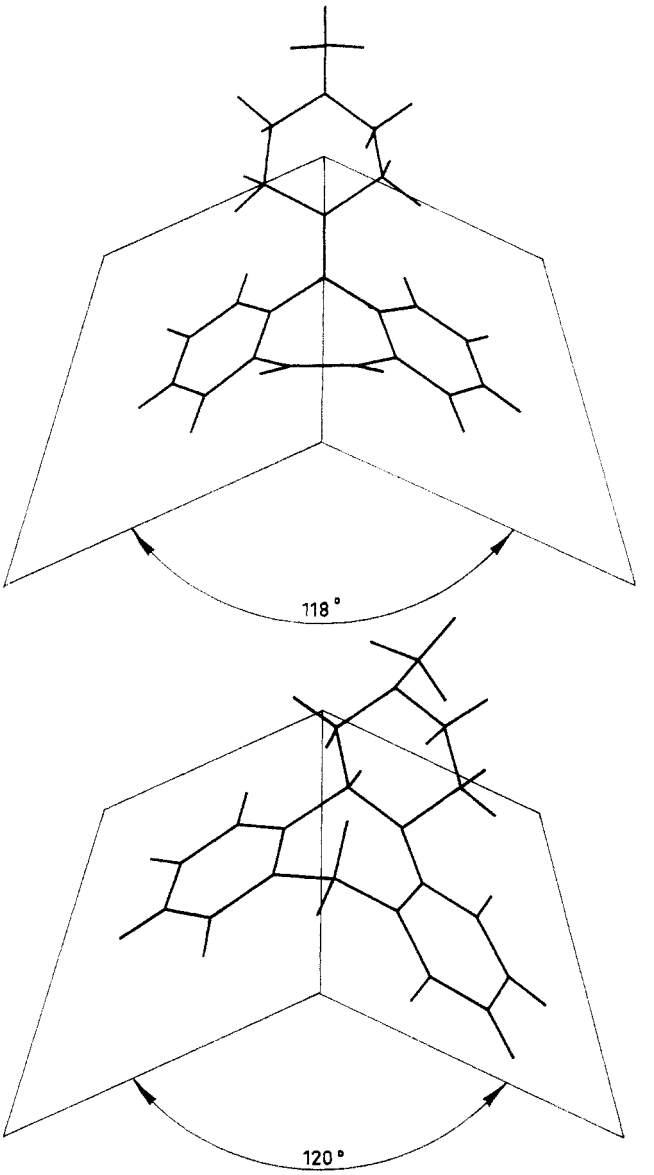


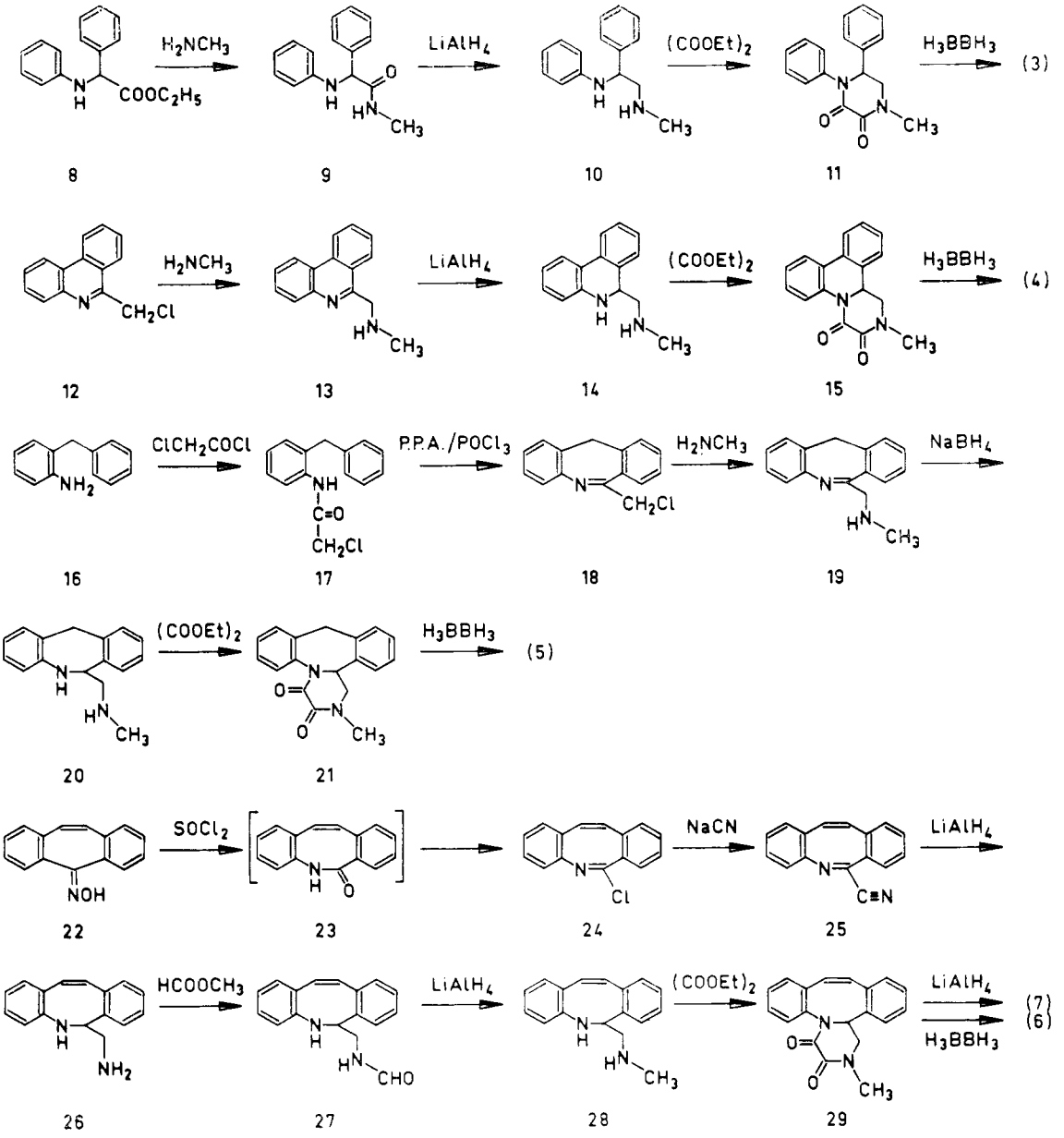
Figure 1.—The projection of Dreiding models of cyproheptadine (upper model) and mianserin (lower model) shows the angles made by the planes of the benzene rings.

Discussion

From the difference in activity between **4**, **6**, and **7** on the one hand, and **5** on the other hand it would appear that, apart from the rigidity of the molecule, other factors may play a part. Inspection of Dreiding models of cyproheptadine and mianserin shows that the intersection angles between the planes formed by the aromatic nuclei are very similar, being about 118 and 120°, respectively (Figure 1). For **4** and **7** these angles are very different, *viz.*, 180 and 58°, whereas **6** is a flexible molecule.

It seems reasonable to assume that in the present type of compounds an angle of about 120° is a structural requirement for high antiserotonin activity.

SCHEME I



Experimental Section

Evaporations were conducted under reduced pressure using a Büchi Rotavapor evaporator. Melting points are not corrected and were taken on a Büchi capillary melting point apparatus. Microanalyses were crried out by H. Pieters and W. J. Buis, Laboratory of Organic Chemistry of the University of Amsterdam, The Netherlands. For the nomenclature of the fused heterocyclic ring systems were used: rule B-3 and rule A-21 of the IUPAC "Nomenclature of Organic Chemistry," Section A and B, 2nd ed, 1966. The melting points and formulas for the compounds are given in Table II.

2-Methyl-1,3,4,15b-tetrahydro-2H-dibenzo[*c,g*]pyrazino[1,2-*a*]-azocine (7).—To a suspension of 2 g of LAH (0.053 mole) in 300 ml of Et_2O, 2 g (0.007 mole) of 2-methyl-3,4-diketo-1,3,4,15b-tetrahydro-2H-dibenzo[*c,g*]pyrazino[1,2-*a*]azocine (**29**) was added The mixture was boiled for 15 hr under N_2. After cooling to 0° 8 ml of H_2O was added dropwise while stirring. The mixture was filtered and the filtrate was evaporated to dryness to give 1.75 g of a yellow oil. Chromatography over silica gel and elution with MeOH–$(CH_3)_2CO$ (1:1) yielded 1.05 g (58%) of the pure piperazino compound. It was dissolved in Et_2O and treated with a solution of maleic acid in EtOH to give the crystalline maleate, 0.7 g (27%), slightly pink needles, mp 160–162°. The analytical sample, mp 161–162°, was obtained by recrystallization from EtOH. *Anal.* ($C_{23}H_{24}N_2O_4$) C, H, N.

2-Anilino-2-phenyl-N-methylacetamide (9).—To 350 ml of a 20% solution of $MeNH_2$ in MeOH was added 45 g (0.18 mole) of ethyl 2-phenyl-2-anilinoacetate[8] (**8**). The mixture was stirred until all crystals were dissolved. After standing at room temperature for 3 days the solution was evaporated to dryness and the solid residue was crystallized from MeOH to give 33 g (87%) of amide, mp 110–112°. The analytical sample melted at 124–125° (from MeOH). *Anal.* ($C_{15}H_{16}N_2O$) C, H, N.

1-Phenyl-1-anilino-2-methylaminoethane (10).—To a suspension of 24 g (0.1 mole) of **9** in 500 ml of anhydrous Et_2O 12 g (0.3 mole) of LAH was added and the mixture was refluxed for 4 hr. After cooling, excess hydride was decomposed with vigorous stirring by slow addition of 48 ml of H_2O. The inorganic products were removed by filtration. The filtrate was evaporated to dryness to give 22 g (97%) of a yellow oil. A part of the product was converted to its hydrochloride. The analytical sample melted at 230–232° (from EtOH). *Anal.* ($C_{15}H_{19}ClN_2$) C, H, N.

6-Methylaminomethylphenanthridine (13).—6-Chloromethylphenanthridine[9] (120 g, 0.55 mole) was added in portions to $MeNH_2$ (2000 ml) at −10°. The solution was kept at this temperature for 2 hr. Excess $MeNH_2$ was removed by distillation *in vacuo* giving a mixture of $MeNH_2 \cdot HCl$ and 6-methylaminomethylphenanthridine. This was triturated with C_6H_6 and

(8) M. Henze, *Chem. Ber.*, **32**, 3056 (1899).
(9) G. T. Morgan and L. P. Walls, *J. Chem. Soc.*, 2447 (1931).

TABLE II

Compd	Mp, °C	Formula	Analyses
3	224–226	$C_{17}H_{22}Cl_2N_2$[a]	C, H, N, Cl
4	236–240	$C_{17}H_{19}ClN_2$[b]	C, H, N, Cl
5	282–284	$C_{18}H_{21}ClN_2$[b]	C, H, N, Cl
6	305–310	$C_{16}H_{23}ClN_2$[b]	C, H, Cl, N
7	161–162	$C_{23}H_{24}NO_4$[c]	C, H, N
9	124–125	$C_{15}H_{16}N_2O$	C, H, N
10	230–232	$C_{15}H_{15}ClN_2$[b]	C, H, N
11	177–179	$C_{17}H_{16}N_2O_2$	C, H, N
13	146–148	$C_{10}H_{18}N_2O_4$[c]	C, H, N
14	169–172	$C_{19}H_{20}N_2O_4$[c]	C, H, N
15	227–229	$C_{17}H_{14}N_2O$	C, H, N
17	119–120	$C_{15}H_{14}ClNO$	C, H, N, Cl
18	138–139	$C_{15}H_{12}ClN$	C, H, N
19	154–157	$C_{20}H_{20}N_2O_4$[c]	C, H, N
20	92–94	$C_{16}H_{18}N_2$	C, H, N
21	245–247	$C_{15}H_{16}N_2O_2$	C, H, N
25	135–136	$C_{16}H_{10}N_2$	C, H, N
26	140–141	$C_{16}H_{16}N_2$	C, H, N
28	117–119	$C_{17}H_{18}N_2$	C, H, N
29	201–206	$C_{19}H_{16}N_2 \cdot 0.5H_2O$[d]	C, H, N

[a] Dihydrochloride. [b] Hydrochloride. [c] Maleate. [d] Hemihydrate.

TABLE III

Compound	Yield, %	Mp, °C	Formula[a]
1-Methyl-4,5-diphenyl-2,3-diketopiperazine (**11**)	60	177–179	$C_{17}H_{16}N_2O_2$
2-Methyl-3,4-diketo-1,3,4,13b-tetrahydro-2H-pyrazino[1,2-*f*]-phenanthridine (**15**)	52	227–229	$C_{17}H_{14}N_2O_2$
2-Methyl-3,4-diketo-1,2,3,10,14b-hexahydro-pyrazino[1,2-*f*]-morphanthridine (**21**)	72	245–247	$C_{18}H_{18}N_2O_2$
2-Methyl-3,4-diketo-1,3,4,15b-tetrahydro-2H-dibenz[*c,g*]pyrazino-[1,2-*a*]azocine (**29**)	49	201–206	$C_{19}H_{16}N_2 \cdot 1.2H_2O$

[a] All compounds analyzed correctly for C, H, N.

washed (5% NaOH, H_2O). After drying (Na_2SO_4) and evaporation of the solvent 107 g (97%) of an oil was obtained which was used directly for the next step (preparation of **14**). A small sample was converted to the maleate which was recrystallized from EtOH to give the analytically pure compound, mp 146–148°. *Anal.* ($C_{19}H_{20}N_2O_4$) C, H, N.

6-Methylaminomethyl-5,6-dihydrophenanthridine (14).—To a suspension of 50 g (1.3 moles) of LAH in 250 ml of dry Et_2O was added slowly a solution of 107 g (0.5 mole) of **13** in 750 ml of Et_2O. The mixture was boiled for 1.5 hr. After cooling in an ice bath excess hydride was decomposed with 200 ml of H_2O. Filtration and evaporation of the solvent yielded 99 g (91%) of a yellow syrup that could not be crystallized. It was used for the next step, the condensation with diethyl oxalate. An analytical sample of the maleate, crystallized from EtOH, melted at 169–172°. *Anal.* ($C_{19}H_{20}N_2O_4$) C, H, N.

2-Benzylchloroacetanilide (17).—To a suspension of 200 g (1.1 moles) of 2-benzylaniline[10] (**16**) in 1 l. of C_6H_6 pyridine (100 ml) was added and the mixture was cooled to 10°. A solution of 95 ml (135 g, 1.2 moles) of chloroacetyl chloride in 125 ml of C_6H_6 was added while stirring, keeping the temperature at 10–12°. After stirring for 0.5 hr at room temperature 160 ml of H_2O was added and stirring was continued for an additional 1 hr. The crystals were filtered, washed (H_2O), and dried at 50°. The C_6H_6 layer of the filtrate was washed (H_2O), dried (Na_2SO_4), and evaporated to dryness. The total crude product was crystallized from EtOH to give 240 g (85%) of **17**, mp 117–119°. The analytical sample (from EtOH) melted at 119–120°. *Anal.* ($C_{15}H_{14}ClNO$) C, H, N, Cl.

6-Chloromethylmorphanthridine (18).—A mixture of 200 g (0.77 mole) of **17**, 250 ml of $POCl_3$, and 1450 g of polyphosphoric acid was heated slowly to 120° and kept at this temperature for 2 hr, upon which it was poured into 7 l. of ice–water. After stirring for 1 hr the mixture was extracted several times with C_6H_6. The combined extracts were washed (H_2O), dried (Na_2SO_4), and concentrated *in vacuo* to 500 ml. After standing overnight the first crop was obtained. Subsequent concentrations of the mother liquor yielded 140 g (75%) of crystalline material, mp 136–138°. The analytical sample (from EtOH) melted at 138–139°. *Anal.* ($C_{15}H_{12}ClN$) C, H, N.

6-Methylaminomethylmorphanthridine (19).—The conversion of 300 g (1.25 moles) of **18** into **19** was effected in the same way as is described for the phenanthridine analog (**13**). There was obtained 261 g (89.5%) of impure product. A small quantity was converted to its maleate, the analytical sample of which melted at 154–157°. *Anal.* ($C_{20}H_{20}N_2O_4$) C, H, N.

6-Methylaminomethyl-5,6-dihydromorphanthridine (20).—In 45 min 151 g (3.0 moles) of $NaBH_4$ was added to a solution of 300 g (1.26 moles) of **19** in 3 l. of EtOH (96%) of 0–5°. Foaming was suppressed by addition of some hexane. The solution was stirred under N_2 at 10–15° for 1.5 hr. Then 6 l. of 2 *N* AcOH was added slowly below 20°. After 40 min 240 ml of glacial AcOH was added with stirring and the mixture was poured into 9 l. of H_2O. The solution was washed three times with 3 l. of Et_2O in order to remove nonbasic by-products. The resulting clear aqueous solution was made alkaline with 1.2 l. of 25% NH_4OH. The dihydro derivative crystallized slowly. It was filtered, washed (H_2O), and dried *in vacuo* to give 240 g (78%) of **20**, mp 80–85°. An analytical sample, mp 92–94°, was obtained by repeated crystallization from EtOH. *Anal.* ($C_{16}H_{18}N_2$) C, H, N.

6-Cyanodibenzo[*b,f*]azocine (25).—To a solution of 50 ml of $SOCl_2$ in 250 ml of C_6H_6 was added 10 g (0.045 mole) of 5H-dibenzo[*a,d*] cyclohepten-5-one oxime[11] (**22**). The mixture was boiled for 16 hr and then evaporated to dryness. The residue was dissolved in dry C_6H_6; the solution was then again evaporated to dryness. The crude 6-chlorodibenzo[*b,f*]azocine (**24**) obtained was directly used for the next step. To this end it was dissolved in 75 ml of DMF, 2.7 g of dry NaCN was added, and the mixture was heated with stirring on a steam bath for 16 hr. After cooling and filtering the solvent was removed by distillation *in vacuo*. The residue solidified on standing. The crystalline mass was digested with MeOH and filtered to yield 7.48 g (72%) of **25**, yellow prisms, mp 130–135°. An analytial sample was obtained by recrystallization from MeOH, mp 135–136°. *Anal.* ($C_{16}H_{10}N_2$) C, H, N.

6-Aminomethyl-5,6-dihydrodibenzo[*b,f*]azocine (26).—A solution of 5 g (0.022 mole) of **25** in 50 ml of dry THF was added dropwise to a suspension of 5 g (0.13 mole) of LAH in 100 ml of dry THF. The mixture was boiled for 24 hr and cooled to 0° and 20 ml of H_2O was added while stirring. The inorganic products were removed by filtration and the filtrate was evaporated to dryness, leaving 4.8 g (93%) of the crude amine. Crystallization of the compound from C_6H_6 gave an analytical sample, mp 140–141°. *Anal.* ($C_{16}H_{16}N_2$) C, H, N.

6-Formamidomethyl-5,6-dihydrodibenzo[*b,f*]azocine (27).—To a solution of 18.5 g (0.078 mole) of **26** in 50 ml of MeOH was added 180 ml of freshly distilled methyl formate. The mixture was boiled for 20 hr under N_2. The solvents were removed by distillation to give 20.7 g (100% of the formamido compound as a yellow oil. It was used for the next step without further purification.

6-Methylaminomethyl-5,6-dihydrodibenzo[*b,f*]azocine (28).—A solution of 21 g (0.08 mole) of **27** in 50 ml of dry THF was added slowly while stirring to a suspension of 11 g (0.29 mole) of LAH in 200 ml of THF and 400 ml of Et_2O was added. The mixture was boiled for 16 hr under N_2. After cooling to 0° 44 ml of H_2O was added dropwise and stirring was continued for an additional 1 hr. The inorganic products were removed by filtering and the filtrate was evaporated to dryness. The solid residue was triturated with hexane and filtered to yield 17.2 g (87%) of **28**, mp 111–114°. An analytical sample, mp 117–119°, was obtained by crystallization from C_6H_6–hexane. *Anal.* ($C_{17}H_{18}N_2$) C, H, N.

Preparation of the Substituted Diketopiperazines (11, 15, 21, 29) by the Riebsomer Reaction.—A mixture of 1 mole of the diamine **10**, **14**, **20**, or **28** and 1.2 moles of diethyl oxalate was

(10) C. L. Hewett, L. J. Lermit, H. T. Openshaw, A. R. Todd, A. H. Williams, and F. N. Woodward, *J. Chem. Soc.*, 292 (1948).

(11) C. van der Stelt, W. J. Heuss, and W. Th. Nauta, *Arzneimittel-Forsch.*, **14**, 116 (1964).

TABLE IV

Compound	Salt	Yield, %	Mp, °C	Formula	Analyses
1-Methyl-4,5-diphenylpiperazine (**3**)	2HCl	85	224–226	$C_{17}H_{22}C_2N_2$	C, H, N
2-Methyl-1,3,4,13b-tetrahydro-2H-pyrazino[1,2-*f*]-phenanthridine (**4**)	HCl	92	236–240	$C_{17}H_{19}ClN_2$	C, H, N, Cl
2-Methyl-1,2,3,4,10,14b-hexahydropyrazino[1,2-*f*]-morphanthridine (**5**)	HCl	90	282–284	$C_{18}H_{21}ClN_2$	C, H, N, Cl
2-Methyl-1,3,4,10,11,15b-hexahydro-2H-dibenz[*c*,*g*]-pyrazino[1,2-*a*]azocine (**6**)	HCl	43	305–310	$C_{19}H_{23}ClN_2$	C, H, N, Cl

heated during 45 min from room temperature to 140°. The temperature was then raised to 180° in 15 min and kept at this level for 0.5 hr. During the reaction about 50 ml of EtOH distilled off. The reaction mixture was cooled and treated with 200 ml of C_6H_6. The crystals were filtered, washed with Et_2O, and dried *in vacuo* to give the diketopiperazino compounds listed in Table III.

Diborane Reduction of the Substituted Diketopiperazines 11, 15, 21, and 29 to the Piperazino Compounds 3–6.—To a suspension of 120 g (3.2 moles) of $NaBH_4$ in 350 ml of dry THF was added dropwise a solution of 520 ml of BF_3 etherate during 2 hr. The generated B_2H_6 was introduced directly with stirring into a suspension of 100 g of the diketopiperazino compound. The entire manipulation was carried out under N_2. The mixture was then refluxed for 1 hr. Excess B_2H_6 was decomposed by adding slowly 350 ml of 96% EtOH, and the solution was evaporated to dryness. The vitreous residue was dissolved in 1800 ml of 18% aqueous HCl and heated on a steam bath for 1 hr, cooled, made alkaline with 30% NaOH, and extracted with CH_2Cl_2. The extract was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness to give 90–95% of the oily piperazino compound. The product was converted to its hydrochloride with alcoholic HCl and recrystallized from EtOH. In this way the compounds listed in Table IV were obtained.

Effect of Eight Prostaglandins on Platelet Aggregation[1]

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Eight prostaglandins, PGE_1, PGE_2, PGA_1, PGA_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, $PGF_{1\beta}$, and $PGF_{2\beta}$, were tested for their effect on platelet aggregation–adhesion induced by ADP, thrombin, and collagen. All compounds inhibited aggregation in platelet-rich rat plasma and human plasma to varying degrees. PGE_1 was the most active compound in the group. In addition, PGE_1 showed very potent thrombolytic effect against ADP-induced platelet thrombi *in vitro*. A single intravenous injection of 3 mg of PGE_1/kg in rats inhibited platelet aggregation in blood samples withdrawn from animals 30 min following the injection. Platelet aggregation was also inhibited significantly in rats given infusions of PGE_1 at 1.8 mg/kg per day for 30 days.

Prostaglandins are powerful vasoactive compounds which occur in human seminal plasma, sheep seminal vesicles, and other tissues. Born and coworkers[3] have reported that a number of vasoactive compounds show a corresponding ability to affect platelet behavior. Since prostaglandin E_1 (PGE_1) is known to be a potent vasodilator, it was of interest to investigate the influence of PGE_1 and other prostaglandins on platelet aggregation. Kloeze[4] first reported that PGE_1 inhibits ADP-induced aggregation as well as glass adhesion of platelets, whereas PGE_2 accelerates platelet aggregation. Weeks, Sekhar, and DuCharme[5] have reported the comparative activity of prostaglandins E_1, A_1, E_2, and A_2 on aggregation of rabbit platelets. The present paper deals with the influence of eight structurally related prostaglandins on adenosine diphosphate (ADP), thrombin (recalcified plasma), and collagen induced aggregation of human and rat platelets.

Experimental Section

Prostaglandins.—The following prostaglandins have been studied: 11α,15(*S*)-dihydroxy-9-oxo-13-*trans*-prostenoic acid (PGE_1), 11α,15(*S*)-dihydroxy-9-oxo-5-*cis*,13-*trans*-prostadienoic acid (PGE_2), 15(*S*)-hydroxy-9-oxo-10,13-*trans*-prostadienoic acid (PGA_1), 15(*S*)-hydroxy-9-oxo-5-*cis*-10,13-*trans*-prostadienoic acid (PGA_2), 9α,11α,15(*S*)-trihydroxy-13-*trans*-prostenoic acid ($PGF_{1\alpha}$), 9α,11α,15(*S*)-trihydroxy-5-*cis*,13-*trans*-prostadienoic acid ($PGF_{2\alpha}$), 9β,11α,15(*S*)-trihydroxy-13-*trans*-prostenoic acid ($PGF_{1\beta}$), 9β,11α,15(*S*)-trihydroxy-5-*cis*,13-*trans*-prostadienoic acid ($PGF_{2\beta}$). The purity of all prostaglandins was checked by tlc.

Inhibition of Platelet Aggregation *in Vitro*.—Chandler's revolving plastic loop technique as modified by Silver[6] was used for the study of platelet aggregation and thrombus formation. Blood from the abdominal aorta of normal male rats (Sprague–Dawley, Spartan strain) was drawn into a plastic syringe containing sodium citrate solution (1 part of 3.8% sodium citrate solution to 9 parts of blood), thoroughly mixed by gentle tilting of the syringe, and centrifuged at 1200 rpm for 10 min. The supernatant platelet-rich plasma was collected by siliconized Dispo pipets. This platelet-rich rat plasma (PRRP, 0.8 ml) was transferred into a plastic loop (Transflex tubing No. 8, 3M Company) by a plastic syringe and 0.1 ml of 0.85% saline was added. The loop was closed with a short segment of another plastic tubing (Transflex tubing No. 6), mounted on a disk, and rotated at 12 rpm for 1 min. One tenth milliliter of 0.25 *M* $CaCl_2$ solution was injected through the loop onto the plasma layer; four stop watches and the loop were started simultaneously. Four successive end points were timed: aggregation of

(1) (a) A preliminary report of this work was presented orally at the Third Platelet Conference, Oak Ridge, Tenn., June 1967; (b) N. Chandrasekhar, *Blood*, **30**, 554 (1967).

(2) Formerly N.Chandrasekhar.

(3) G. V. R. Born, R. J. Haslam, N. Goldman, and R. D. Lowe, *Nature*, **205**, 678 (1965).

(4) J. Kloeze, Nobel Symposium 2, Prostaglandins, S. Bergström and B. Samuelsson, Ed., Almquist and Wiksell, Stockholm, 1967, p 241.

(5) J. R. Weeks, N. C. Sekhar, and D. W. DuCharme, *J. Pharm. Pharmacol.*, **21**, 103 (1969).

(6) M. J. Silver, *Am. J. Physiol.*, **209**, 1128 (1965).