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Lysophosphatidylethanolamine and 2-Desoxylysophosphatidylethanolamine Derivatives. 1. Potential Renin Inhibitors

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A wide variety of derivatives of phospholipids related to natural phosphatidylethanolamines were prepared and evaluated as potential renin inhibitors in two *in vitro* assays. Lysophosphatidylethanolamines and 2-desoxylysophosphatidylethanolamines were also synthesized and evaluated. None of the new compounds were more active than a phospholipid mixture isolated from hog kidney but two compounds, **6g** and **6j**, which contain the 1-adamantyl moiety, showed comparable activity in both assays. In this series the lyso and 2-desoxy derivatives were generally active at comparable concentrations regardless of the hydrophobic group. General procedures for the preparation of ethanolamine derivatives are also described.

A phospholipid isolated from dog,[1] hog,[1] rat,[2] and human[3,4] was reported to be a natural precursor of a renin inhibitor. This inhibitor was said to repress the action of renin *in vitro*,[1] inhibit the *in vitro* response to injected renin in bilaterally nephrectomized rats,[5] and reduce the blood pressure of rats with acute and chronic renal hypertension by 70–130 mm when dosed daily (from 4 to 12 days) by im (3.5 mg/kg), oral (150 mg per 250–350 g rat), or infusion (5 mg/kg) routes.[6] The phospholipid had no effect on the blood pressure of normotensive rats. The "active" inhibitor was derived from the phospholipid by phospholipase hydrolysis and was provisionally identified as a lysophosphatidyl amino acid derivative.[1] A later report[2] suggested that the preinhibitor, isolated from different species and different tissue, was a phosphatidylethanolamine with a unique fatty acid complement.

Our interest in phospholipids[7] and their involvement in enzymatic reactions[8] led us to investigate the potential for development of a phospholipid renin inhibitor as an effective mediator of the renin–angiotensin–aldosterone system. We isolated a phospholipid from hog kidney, according to the published procedure,[1] which, as reported, inhibited the conversion of renin substrate to angiotensin I in two *in vitro* assays. This substance was found to be a mixture of many molecular species of phosphatidylethanolamine esterified with a relatively high proportion of polyunsaturated fatty acids. Stearate, oleate, and linoleate were esterified predominately at the primary hydroxyl, and almost half of the ester at the secondary hydroxyl was arachidonate with lesser amounts of long-chain polyunsaturated fatty acids.[9]

For this study, a series of synthetic phospholipids and phospholipid-like derivatives were prepared and their renin-inhibitory activity evaluated in two *in vitro* test systems. It was anticipated that once structure–activity relationships had been investigated, inhibitory phospholipids could be prepared which would be stable to natural hydrolytic enzymes and thus be potentially useful for *in vivo* studies. Potential inhibitors have been prepared including the phosphatidylethanolamine derivatives **6**, lysophosphatidylethanolamines **7**, 2-desoxylysophosphatidylethanolamines **11** and **13**, and other related compounds.

Chemistry.—Compounds **6** were prepared either by the condensation of the Ag salt of the phosphate **2**[10] with the I derivatives **1** (Scheme I, method A), or by the phosphorylation of a 1,2-diglyceride **3** with the dichlorophosphate **4**[7] (method B). Successive acylations[11] of 3-iodopropane-1,2-diol with the appropriate acid chlorides gave compounds **1** (Table I). Compounds **3** were synthesized by a simplified method which is applicable to the preparation of optically active, unsaturated 1,2-diglycerides[8] (Scheme II, Tables II and III). The protected intermediates **5a** and **5b** were not isolated; treatment of **5a** at 0° with dry HCl[10] or reaction of **5b** with Zn in AcOH[7] afforded the synthetic phosphatidylethanolamines **6** (Table IV). Enzymatic hydrolysis of **6** with phospholipase A from snake venom[12] gave the lysophosphatidylethanolamines **7** which were not purified, but were used directly in the assays.

As activity resided in the lyso derivatives **7** (compounds **6** were generally inactive—see Biological Evaluation below) it was of interest to prepare the 2-desoxylysophosphatidylethanolamine derivatives, the esters **11** and ethers **13**, which are related to **7** but are devoid of the 2-OH group. Previously, it was shown that other desoxyphospholipids were active in certain test systems. Desoxylysophosphatidyl choline derivatives have anticlotting activity[13] and are cleaved by lipase and phospholipase preparations.[14] Desoxylysophosphatidyl glycerols exhibited some activity in the

(1) S. Sen, R. R. Smeby, and F. M. Bumpus, *Biochemistry*, **6**, 1572 (1967).
(2) D. H. Osmond, R. R. Smeby, and F. M. Bumpus, *J. Lab. Clin. Med.*, **73**, 795 (1969).
(3) D. Ostrovsky, S. Sen, R. R. Smeby, and F. M. Bumpus, *Circ. Res.*, **21**, 497 (1967).
(4) D. H. Osmond, L. A. Lewis, R. R. Smeby, and F. M. Bumpus, *J. Lab. Clin. Med.*, **73**, 809 (1969).
(5) S. Sen, R. R. Smeby, and F. M. Bumpus, *Amer. J. Physiol.*, **214**, 337 (1968).
(6) R. R. Smeby, S. Sen, and F. M. Bumpus, *Circ. Res.*, **21**, Suppl., II, II-129 (1967).
(7) F. R. Pfeiffer, S. R. Cohen, and J. A. Weisbach, *J. Org. Chem.*, **34**, 2795 (1969).
(8) F. R. Pfeiffer, C. K. Miao, and J. A. Weisbach, *ibid.*, **35**, 221 (1970).
(9) F. R. Pfeiffer, unpublished results.
(10) F. J. M. Daeman, *Chem. Phys. Lipids*, **1**, 476 (1967).
(11) G. H. de Haas and L. L. M. van Deenen, *Recl. Trav. Chim. Pays-Bas*, **80**, 951 (1961).
(12) C. Long and I. F. Penny, *Biochem. J.*, **65**, 382 (1957).
(13) H. Kaneko and I. Hara, *Bull. Soc. Chim. Biol.*, **46**, 895 (1964); *Chem. Abstr.*, **62**, 2951*h* (1965).
(14) H. Eibl and O. Westphal, *Justus Liebigs Ann. Chem.*, **709**, 244 (1967), and ref cited therein.

SCHEME I

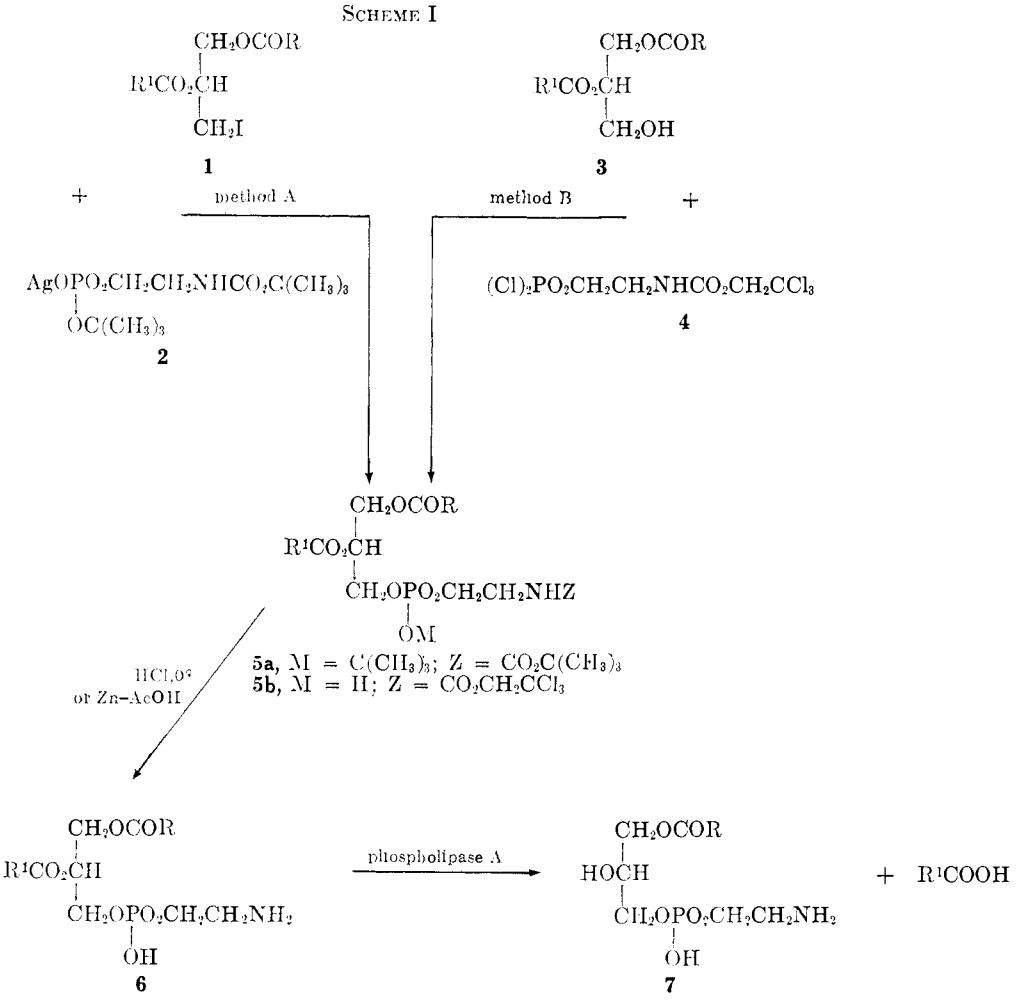
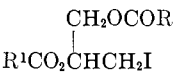


TABLE I

1,2-DIACYL-3-DESOXY-3-IODO-*sn*-GLYCEROLS

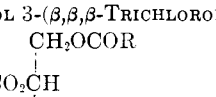


No.	R[a]	R[1]	Yield, %	$[\alpha]^{25}D$, deg (*c*, %)[b]	Formula[c]
1a	$CH_3(CH_2)_{16}$	$CH_2{=}CH(CH_2)_8$	78[d]	+5.1 (1.04)	$C_{32}H_{59}IO_4$
1b	$CH_2{=}CH(CH_2)_8$	$CH_2{=}CH(CH_2)_8$	89	+4.7 (0.90)	$C_{25}H_{43}IO_4$
1c	1-Adamantyl	$CH_2{=}CH(CH_2)_8$	65	+2.9 (0.89)	$C_{25}H_{39}IO_4$
1d	1-Adamantyl	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[f]	72	+0.8 (1.02)	$C_{37}H_{53}IO_4$
1e	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[f]	1-Adamantyl	41	+3.7 (0.88)	$C_{32}H_{53}IO_4$
1f	1-Adamantyl	1-Adamantyl	67[d]	+0.7 (1.14)	$C_{25}H_{35}IO_4$
1g	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[e]	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[e]	61	+3.1 (0.78)	$C_{39}H_{71}IO_4$

[a] The 1-acyl-3-desoxy-3-iodo precursors of 1a, 1c, 1d, and 1e were prepd by reported procedures [see G. H. de Haas and L. L. M. van Deenen, *Recl. Trav. Chim. Pays-Bas*, **80**, 951 (1961)]: 1-octadecanoyl-3-desoxy-3-iodo-*sn*-glycerol, mp 64–66° (from Et_2O–MeOH); $[\alpha]^{25}D$ +1.62° (*c* 1.0, $CHCl_3$) [lit.[a] mp 65–66°; $[\alpha]^{20}D$ +1.8° (*c* 10, $CHCl_3$)]; 1-(1-adamantoyl)-3-desoxy-3-iodo-*sn*-glycerol, mp 63–65° (from cyclohexane-petr ether); $[\alpha]^{25}D$ +1.79° (*c* 1.14, $CHCl_3$) [*Anal.* ($C_{13}H_{21}IO_3$) C, H, I]; 1-(*cis*-9-octadecenoyl)-3-desoxy-3-iodo-*sn*-glycerol, oil, $[\alpha]^{25}D$ +1.65° (*c* 1.1, $CHCl_3$) [lit.[a] mp 33.4°; $[\alpha]^{20}D$ +1.9° (*c* 10, $CHCl_3$)]. [b] Optical rotations were detd in $CHCl_3$. [c] All compds were anal. for C, H, I. [d] All compds are oils except 1a, mp 26–28° (from MeOH), and 1f, mp 108–110° (from Et_2O–MeOH). [e] Double bond is trans. [f] Double bond is cis.

TABLE II

1,2-DIACYL-*sn*-GLYCEROL 3-(β,β,β-TRICHLOROETHYL) CARBONATES

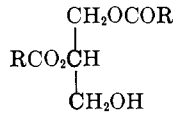


No.	R	Yield, %	Recrystn solvent	Mp, °C	$[\alpha]^{25}D$, deg (*c*, %)[a]	Formula[b]
9a	$CH_3(CH_2)_{16}$	75	Et_2O–MeOH	56–57	−1.7 (1.05)	$C_{42}H_{77}Cl_3O_7$
9b	1-Adamantyl	81	MeOH	117–118.5	−0.7 (1.03)	$C_{28}H_{37}Cl_3O_7$
9c	1-Adamantylmethyl	72		Oil	−5.4 (0.92)	$C_{30}H_{41}Cl_3O_7$
9d	Dihydrophytyl[c]	34		Oil	−1.4 (0.54)	$C_{46}H_{85}Cl_3O_7$

[a] Optical rotations were detd in $CHCl_3$. [b] All compds were anal. for C, H, Cl. [c] Dihydrophytyl = 3, 7, 11, 15-tetramethylhexadecyl.

TABLE III

1,2-DIACYL-*sn*-GLYCEROLS

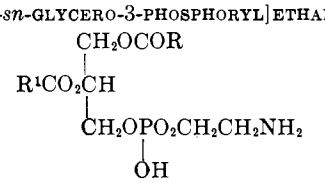


No.	R	Yield, %	Recrystn solvent	Mp, °C	$[\alpha]^{25}D$, deg (c, %)[a]	Formula[b]
3a	$CH_3(CH_2)_{16}$	87	Hexane	74–74.5[c]	−2.6 (1.0)	$C_{39}H_{76}O_5$
3b	1-Adamantyl	90	Hexane	98.5–100	−0.4 (1.1)	$C_{25}H_{36}O_5$
3c	1-Adamantylmethyl	91		Oil	−0.7 (0.9)	$C_{27}H_{40}O_5$
3d	Dihydrophytyl[d]	82		Oil	−1.5 (1.2)	$C_{43}H_{84}O_5$

[a] Optical rotations were detd in $CHCl_3$. [b] All compds anal. for C, H. [c] J. C. Sowden and H. O. L. Fischer [*J. Amer. Chem. Soc.*, **63**, 3244 (1941)] reported mp 74–74.5°, $[\alpha]D$ −2.7° (*c* 6.18, $CHCl_3$). [d] See footnote *c* of Table II.

TABLE IV

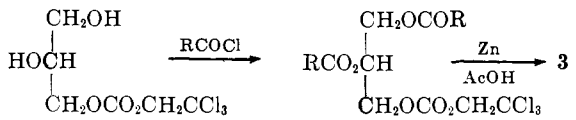
O-[1,2-DIACYL-*sn*-GLYCERO-3-PHOSPHORYL]ETHANOLAMINES



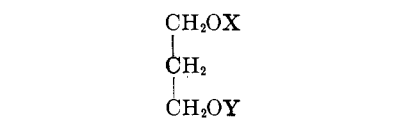
No.	R	R^1	Method[a]	Yield,[b] %	Mp, °C[c]	$[\alpha]^{25}D$, deg (c, %)[d]	Formula[e]
6a	$CH_3(CH_2)_{16}$	$CH_3(CH_2)_{16}$	B	48	204[f]	+6.0 (1.0)	$C_{41}H_{82}NO_8P$[g]
6b	$CH_3(CH_2)_{16}$	$CH_2{=}CH(CH_2)_8$	A	51	206–208	+7.1 (1.0)	$C_{34}H_{66}NO_8P$
6c	$CH_2{=}CH(CH_2)_8$	$CH_2{=}CH(CH_2)_8$	A	38	210–212	+14.8 (0.5)	$C_{27}H_{50}NO_8P$
6d	1-Adamantyl	$CH_2{=}CH(CH_2)_8$	A	30	197–199	+7.8 (0.5)	$C_{27}H_{46}NO_8P$[h]
6e	1-Adamantyl	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[o]	A	42	Wax	+13.8 (1.0)	$C_{34}H_{60}NO_8P$[i]
6f	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[o]	1-Adamantyl	A	33	Wax[k]	+7.1 (0.5)	$C_{34}H_{60}NO_8P$[g]
6g	1-Adamantyl	1-Adamantyl	A	41			
			B	36	207–210	+8.0 (1.0)	$C_{27}H_{42}NO_8P$[j]
6h	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[n]	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$[n]	A	24	196–198	+8.3 (0.9)	$C_{41}H_{78}NO_8P$[i]
6i	Dihydrophytyl[l]	Dihydrophytyl	B	25	Wax[m]	+7.6 (0.8)	$C_{45}H_{90}NO_8P$
6j	1-Adamantylmethyl	1-Adamantylmethyl	B	29	207–208	+10.1 (0.8)	$C_{29}H_{46}NO_8P$[j]

[a] The symbols used in this column are explained in the text. [b] Yields are based on chromatogd products. [c] All compds were crystd or pptd from $CHCl_3$–Me_2CO, except **6g** (from $CHCl_3$–petr ether) and **6j** (2-butanone–MeCN). [d] Optical rotations detd in $CHCl_3$. [e] All compds were anal. for C, H, N and were dried at 40° (1 mm) for 18 hr. [f] Reported mp 173–175°, $[\alpha]^{24}D$ +6.0° [E. Baer, J. Maurukas, and M. Russell, *J. Amer. Chem. Soc.*, **74**, 152 (1952)]. [g] Hemihydrate. [h] Dihydrate. [i] Hydrate. [j] Sesquihydrate. [k] Purified by thick-layer chromatography on Brinkmann silica gel GF 254 (1 mm) plates using 65:25:4 $CHCl_3$–MeOH–H_2O as the eluent. [l] See footnote *c* of Table II. [m] Purified by column chromatogr on DEAE-cellulose according to the method of G. Rouser, G. Kritchevsky, D. Heller, and E. Lieber, *J. Amer. Oil Chem. Soc.*, **40**, 425 (1963). [n] Double bond is trans. [o] Double bond is cis.

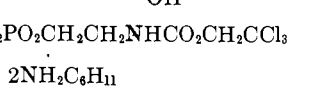
SCHEME II



Wassermann assay.[15] Several 1-acyldesoxylysolecithins caused lysis of red cells.[16]



10, X = COR; Y = H
11, X = COR; Y = $PO_2CH_2CH_2NH_2$ (OH)
12, X = CH_2R; Y = H
13, X = CH_2R; Y = $PO_2CH_2CH_2NH_2$ (OH)



$(HO)_2PO_2CH_2CH_2NHCO_2CH_2CCl_3$

$2NH_2C_6H_{11}$

14

An excess of propane-1,3-diol was acylated with an acid chloride or treated with the appropriate mesylate in DMSO to give, after chromatographic purification, **10** and **12**, respectively (Table V). Compounds **10** were phosphorylated with **4** and converted to **11** with Zn in AcOH. Phosphorylation of the ethers **12** with **4** or **14**[7] in the presence of trichloroacetonitrile[17] (meth-

TABLE V

MONOACYL- (AND ALKYL)PROPANE-1,3-DIOLS

$ROCH_2CH_2CH_2OH$

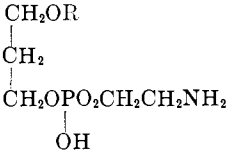
No.	R[a]	Yield, %	Formula[e]
10a	$CO(CH_2)_{16}CH_3$[b]	79	$C_{21}H_{42}O_3$
10b	$CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$[f]	71	$C_{21}H_{40}O_3$
10c	CO(1-adamantyl)[c]	74	$C_{14}H_{22}O_3$
12a	$CH_2(CH_2)_{16}CH_3$[d]	55	$C_{21}H_{44}O_2$
12b	$CH_2(CH_2)_7CH{=}CH(CH_2)_7CH_3$[f]	52	$C_{21}H_{42}O_2$
12c	$CH_2(CH_2)_7(CH{=}CHCH_2)_2(CH_2)_3CH_3$[f]	64	$C_{21}H_{40}O_2$
12d	CH_2CH_2(1-adamantyl)	56	$C_{15}H_{26}O_2$

[a] All compds are oils except where indicated. [b] Mp 49–51° (from Et_2O–MeOH). [c] Mp 39–41° (petr ether). [d] Mp 45–47° (Et_2O–MeOH). [e] All compds were anal. for C, H. [f] Double bond is cis.

(15) K. Inoue and S. Najima, *Chem. Phys. Lipids*, **1**, 360 (1967).
(16) F. C. Reman, R. A. Demel, J. deGier, L. L. M. van Deenen, H. Eibl, and O. Westphal, *ibid.*, **3**, 221 (1969).
(17) F. Cramer, W. Rittersdorf, and W. Böhm, *Justus Liebigs Ann. Chem.*, **654**, 180 (1962).

TABLE VI

O-[1-ACYL (OR -ALKYL) -2-DESOXYLYSOGLYCERO-3-PHOSPHORYL]ETHANOLAMINES



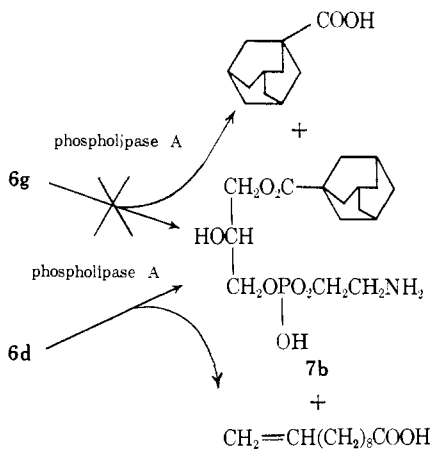
No.	R	Method[a]	Yield,[b] %	Recrystn solvent	Mp, °C	Formula[c]
11a	$CO(CH_2)_{16}CH_3$	B	42	$CHCl_3$–MeOH	202–204	$C_{23}H_{48}NO_6P$[e]
11b	$CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$[h]	B	34	$CHCl_3$–MEK[d]	204–206	$C_{23}H_{46}NO_6P$
11c	CO(1-adamantyl)	B	37	$CHCl_3$–Me_2CO	222–224	$C_{16}H_{28}NO_6P$
13a	$CH_2(CH_2)_{16}CH_3$	C	40	$CHCl_3$–Me_2CO	224–226	$C_{23}H_{50}NO_5P$[e]
13b	$CH_2(CH_2)_7CH{=}CH(CH_2)_7CH_3$[h]	B	30	$CHCl_3$–Me_2CO	206–208	$C_{23}H_{48}NO_5P$[f]
		C	35			
13c	$CH_2(CH_2)_7(CH{=}CHCH_2)_2(CH_2)_3CH_3$[h]	B	28	$CHCl_3$–MEK[d]	193–196	$C_{23}H_{46}NO_5P$[e]
13d	CH_2CH_2(1-adamantyl)	B	40	$CHCl_3$–MeCN	208–210	$C_{17}H_{32}NO_5P$[g]

[a] The symbols used in this column are explained in the text. [b] Yields are based on chromatogd products. [c] All compds were anal. for C, H, N and were dried at 40° (1 mm) for 18 hr. [d] MEK = methyl ethyl ketone. [e] Hemihydrate. [f] Anal. as 0.25hydrate by thermal gravimetric analysis (tga); H_2O: calcd, 0.99; found, 0.6. [g] Quarter hydrate by tga; H_2O: calcd, 1.23; found, 1.68. [h] Double bonds are cis.

od C) gave **13** after removal of the CCl_3CH_2OCO group (Table VI).

The attempted enzymatic preparation of *O*-[1-(1-adamantoyl)-3-*sn*-glycerophosphoryl]ethanolamine (**7b**) from the 1,2-di(1-adamantoyl) derivative **6g** was not successful; **6g** is inert to the phospholipase A in snake venom (*Crotalus adamanteus*). Compound **7b** was readily prepared enzymatically (Scheme III)

SCHEME III



from *O*-[1-(1-adamantoyl-2-(10-undecenoyl)-*sn*-glycerophosphoryl]ethanolamine (**6d**).

Biological Evaluation.—The ability of these compounds to inhibit the renin-catalyzed conversion of renin substrate to angiotensin I was examined in 2 test systems. Renin activity was determined indirectly by measuring the angiotensin I hydrolysis products, angiotensin II and His-Leu. A bioassay and a biochemical assay were used. The bioassay is a modification of a reported procedure[1,3] and involves a two-step sequence: (1) *in vitro* production of angiotensin I by the action of hog renin on hog renin substrate in the presence of a potential inhibitor, and (2) *in vivo* bioassay of the generated angiotensin I in the anesthetized rat[18] indirectly *via* the pressor response elicited by the angiotensin II formed by the rat's converting enzyme. The biochemical assay also involves two enzyme reactions. Renin substrate, in this case a tetradecapeptide,[19] was incubated with renin and the potential inhibitor. The derived angiotensin I was hydrolyzed *in vitro* with a preparation of converting enzyme to give the octapeptide, angiotensin II, and His-Leu. The amount of His-Leu released was measured spectrophotofluorometrically and compared to appropriate controls.

The biological data may be summarized as follows (Table VII). Potential inhibitors were tested at doses of $1–5 \times 10^{-3}$ *M*. Compounds **6g** and **6j**, which are esterified with 1-adamantoyl moieties, showed good activity in both assays. In the bioassay **6c** and **6d** having shorter fatty acyl residues were slightly active. In contrast nearly all of the lyso derivatives **7**, and the 2-desoxylyso derivatives **11** and **13** exhibited some renin-inhibitory activity in both assays. These findings are consistent with literature reports[1,6] which show that the lysophosphatidylethanolamines **7** are the inhibitor substances and the phosphatidylethanolamines **6** are inactive (exceptions noted above) and are precursors of the actual inhibitor **7**. The inactivity of **11a** in the biochemical assay could be attributed to insolubility or incomplete micelle formation. Renin-inhibitory activity does not seem to depend on the degree of unsaturation in the acyl groups, nor is an OH at position 2 necessary. The specific hydrophobic head of the inhibitors does not seem to be as important as the gross hydrophobicity. The ethanolamine moiety seems to be a requirement for inhibition as other phospholipid types were shown to be inactive in a bioassay.[1]

Experimental Section

The compds were routinely checked by ir spectroscopy; melting points were determined in a Thomas-Hoover melting point apparatus and are corrected; optical rotations were taken on a Perkin-Elmer 141 polarimeter. Tlc was carried out on 0.25-mm Anal Tech silica gel GF plates[20] with the solvent systems: 9CE (9:1, v/v, cyclohexane-EtOAc), 3CE (3:1, cyclohexane-EtOAc), and CMW (65:25:4, $CHCl_3$-MeOH-H_2O). The compds were detected by spraying with 40% H_2SO_4 and heating, with ninhydrin, and with a reagent for P.[21] Where analyses are indicated

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TABLE VII

BIOLOGICAL EVALUATION OF POTENTIAL RENIN INHIBITORS[a]

Compd	Bioassay: Concentration ($\times 10^{-3}$ *M*)	Bioassay: Inhibition, %[b]	Biochemical assay: Concentration ($\times 10^{-3}$ *M*)	Biochemical assay: Inhibition, %[b]
6a	2.67	16		
6b	3.08	5		
6c	3.65	26		
6d	3.69	33		
6e	3.02	17		
6f	3.02	18		
6g	3.80	68	1.86	39
			0.93	38
6h	2.69	5		
6i	2.49	5		
6j			1.76	68
			0.88	53
7a, R = $(CH_2)_{16}CH_3$	4.15	69		
7b, R = 1-adamantyl	5.39	61		
7c, R = $(CH_2)_7CH{=}CH(CH_2)_7CH_3$[h]	4.18	66		
7d, R = dihydrophytyl[c]	4.06	45		
11a			2.50	7
			1.25	3
11b	4.33	30		
11c	5.55	64		
13a			2.2	29
			1.1	15
13b	4.36	49		
13d	4.39	56	2.77	47
			1.38	22
6 (from hog kidney)[d]	2.67[f]	5–10	1.33[f]	0
7 (from hog kidney)[e]	4.16[g]	85–95	2.08[g]	85–90

[a] See Experimental Section for detailed descriptions of the assays. [b] Per cent change when compared to controls. [c] See footnote *c* of Table II. [d] Isolated from hog kidney acetone powder (Pentex Biochemicals Division, Miles Laboratories, Kankakee, Ill.) by column chromatogr or thick-layer chromatography on silica gel or DEAE-cellulose. [e] Prepared from the phospholipid isolated from hog kidney by phospholipase A hydrolysis (see Experimental Section). [f] Assuming an average mol wt of about 750. [g] Assuming an average mol wt of about 470. [h] Double bond is cis.

only by symbols of the elements, anal. results obtained for these elements were within ±0.4% of the theoretical values, and were obtained by the Analytical and Physical Chemistry Section of Smith Kline & French Laboratories. Petr ether used had bp 30–60°. Mallinckrodt silica gel (100 mesh) was used for column chromatogr. Nomenclature of the optically active glycerol derivatives follows the recommended rules.[22]

1-(1-Adamantoyl)-2-oleoyl-3-desoxy-3-iodo-*sn*-glycerol (1d) (**Table I**).—A soln of 7.0 g (0.035 mole) of 3-desoxy-3-iodo-*sn*-glycerol[23] in 25 ml of $CHCl_3$ (distd from P_2O_5) and 2.65 ml of anhyd pyridine was cooled to 0° and treated dropwise with 5.8 g (0.029 mole) of 1-adamantane carbonyl chloride[24] in 25 ml of anhyd $CHCl_3$. The mixt was stirred for 18 hr at 25°, dild with 200 ml of Et_2O, and then washed with dil HCl, H_2O, 5% $NaHSO_3$, H_2O, 5% $NaHCO_3$, and H_2O. After being dried (Na_2SO_4), the ether was concd and the product (9.5 g) was chromatogd on 285 g of Florisil using Et_2O–petr ether mixts. A small amt of forerun contained 1,2-di(1-adamantoyl)-3-desoxy-3-iodo-*sn*-glycerol (**1f**), R_f 0.82 (system 9CE). The major product was eluted with Et_2O and the homogeneous cuts were crystd from cyclohexane–petr ether to give 4.8 g (46%) of 1-(1-adamantoyl)-3-desoxy-3-iodo-*sn*-glycerol, mp 63–65°, R_f 0.25 (9CE). *Anal.* ($C_{14}H_{21}IO_3$) C, H, I.

1-(1-Adamantoyl)-3-desoxy-3-iodo-*sn*-glycerol (14.05 g, 0.039 mole) was dissolved in anhyd $CHCl_3$ (30 ml) and anhyd pyridine (2.4 ml) and *cis*-9-octadecenoyl chloride[25] (8.7 g, 0.29 mole) in anhyd $CHCl_3$ (30 ml) was added dropwise with stirring at 0° under N_2. After stirring for 18 hr at room temp the reaction was worked-up as described above and the crude product was chromatogd on Florisil (30 g/g of crude product). The column was developed with petr ether–Et_2O mixts to give oily **1d**, R_f 0.65 (9CE).

1,2-Di(1-adamantoyl)-3-desoxy-3-iodo-*sn*-glycerol (1f).—A soln of 5.72 g (0.0283 mole) of 3-desoxy-3-iodo-*sn*-glycerol, 30 ml of anhyd $CHCl_3$, and 7.5 ml of anhyd pyridine was treated dropwise at 0° with a soln of 12.2 g (0.0614 mole) of 1-adamantane carbonyl chloride, stirred for 18 hr at 25°, and worked-up as described in the previous example. The crude pink oil crystd on standing and was recrystd to give 9.98 g of **1f**.

1,2-Di(1-adamantoyl)-*sn*-glycerol 3-(β,β,β-Trichloroethyl carbonate) (9b) (**Table II**).—1-Adamantanecarbonyl chloride (28.2 g, 0.142 mole) in EtOH-free, anhyd $CHCl_3$ (100 ml) was added dropwise to an ice-cold soln of *sn*-glycerol 3-(β,β,β-trichloroethyl carbonate)[8] (20.0 g, 0.071 mole), anhyd pyridine (14 ml), and anhyd $CHCl_3$ (50 ml). After stirring overnight at room temp, 800 ml of Et_2O was added and the mixt was washed with dil HCl, H_2O, 5% $NaHCO_3$, and H_2O. The dried (Na_2SO_4) ethereal solution was concd to give a colorless oil which was crystd to afford 34.1 g of **9b**, R_f 0.76 (3CE).

1,2-Di(1-adamantoyl)-*sn*-glycerol (3b) (**Table III**).—Compd **9b** (23.2 g, 0.039 mole) was dissolved in 150 ml of 85% AcOH and 200 ml of Et_2O and 48 g of activated Zn[26] was added (slight exotherm). The suspension was stirred at 20–25° for 1 hr and filtered and the filter cake was washed with addnl Et_2O. The filtrate was washed with H_2O (3 × 100 ml), 5% $NaHCO_3$ until neutral, and H_2O again. After drying (Na_2SO_4) the Et_2O was evapd and the oily residue was crystd to yield 14.6 g (90%) of **3b**, mp 96–98°, R_f 0.31 (3CE).

***O*-[1,2-Di(1-adamantoyl)-*sn*-glycero-3-phosphoryl]ethanolamine (6g). Method A** (**Table IV**).—A soln of 1.4 g (0.0028 mole) of **1f** in 200 ml of anhyd C_6H_6 was concd to 100 ml using a Dean–Stark adapter. The soln was cooled slightly and 1.24 g (0.003 mole) of silver *tert*-butyl(*N*-*tert*-butyloxycarbonyl-2-aminoethyl)phosphate[10] (previously dried at 40° *in vacuo* for several hours) was added. The reaction flask was protected from light, and the mixture was refluxed for 2.5 hr. The warm soln was filtered through Supercel to remove AgI, and the filtrate was washed with 5% $NaHCO_3$ and H_2O. The dried ($MgSO_4$) organic layer was concd to give the colorless, oily **5a** (R = R′ = 1-adamantoyl). This was dissolved in dry Et_2O (200 ml) and treated with a stream of dry HCl at 0° for 2 hr. After evapn of the solvent at 30° the residue was dissolved in 30 ml of 4:2:1, v/v, Et_2O–EtOH–H_2O and percolated through a column packed with 30 g of Amberlite IR 45 (OH^-) resin using about 300 ml of addnl solvent. The crude **6g** was obtained on evapn of the solvent at 30°; it was chromatogd on 250 g of a mixture of 2:1, w/w, silica gel and Supercel. The column was developed with 10–50% MeOH in $CHCl_3$. Homogeneous fractions with R_f 0.66 (CMW) were crystd.

***O*-[1,2-Di(1-adamantylacetyl)-*sn*-glycero-3-phosphoryl]ethanolamine (6j). Method B** (**Table IV**).—1,2-Di(1-adamantylacetyl)-*sn*-glycerol (**3c**) (12.8 g, 0.029 mole, freshly prepd from the carbonate **9c** and used without purification) was dissolved in 50 ml of anhyd $CHCl_3$ and 11.5 ml of anhyd pyridine and added dropwise to an ice-cold soln of 15.25 g (0.043 mole) of dichloro(*N*-β,β,β-trichloroethoxycarbonyl-2-aminoethyl)phosphate (**4**)[7] in 50 ml of anhyd $CHCl_3$. The soln was stirred at room temp for 18 hr, dild with 600 ml of Et_2O, and washed with cold dil HCl and brine. The org layer was concd to give crude **5b** (R = R′ = 1-adamantylmethylene). This was dissolved in 25 ml of 90% AcOH and 50 ml of Et_2O and treated with 30 g of activated Zn. The suspension was stirred at 20–25° for 3–4 hr or until tlc (system CMW) indicated complete conversion to the phosphatidylethanolamine **6j**. To the suspension was added solid $NaHCO_3$ (60 g), H_2O (6 ml), and $CHCl_3$ (100 ml). After stirring for 20–30 min, $CHCl_3$ (1 l.) and excess Na_2SO_4 were added and the stirring was continued for another hour. The solvents were filtered and concd, and the residue was chromatogd on 2 kg of 2:1 silica gel–Supercel with 5% MeOH in $CHCl_3$ as the solvent. From the column were obtained a minor product, R_f 0.85 (CMW), formed from excess **4**, and the major product **6j**, R_f 0.69 (CMW), which was crystd.

1-*cis*-9-Octadecenoylpropane-1,3-diol (10b) (**Table V**).—A soln of 5.05 g (0.066 mole) of propane-1,3-diol (dried over Linde 4A molecular sieves), 20 ml of anhyd $CHCl_3$, and 1.6 ml of anhyd

(22) See rules of nomenclature of lipids proposed by the IUPAC–IUB Commission on Biochemical Nomenclature [*Biochemistry*, **6**, 3287 (1967)].

(23) E. Baer and H. O. L. Fischer, *J. Amer. Chem. Soc.*, **70**, 609 (1948). Hydrolysis of the isopropylidene precursor was achieved with 1 *N* HCl in MeOH at 25° for 18 hr.

(24) H. Stetter and E. Rauscher, *Chem. Ber.*, **93**, 1161 (1960).

(25) The acid chlorides were prepared with oxalyl chloride [F. H. Mattson and R. A. Volpenhein, *J. Lipid Res.*, **3**, 281 (1962)].

(26) E. Baer and D. Buchnea, *J. Biol. Chem.*, **230**, 447 (1958).

pyridine was cooled to 0° and a soln of *cis*-9-octadecenoyl chloride (5.0 g, 0.017 mole) in $CHCl_3$ (25 ml) was added dropwise under N_2. The soln was stirred for 18 hr at 25°, dild with Et_2O (150 ml), and washed with dil HCl, H_2O, 5% $NaHCO_3$, and H_2O. Chromatog of the dried Et_2O concn on Florisil (175 g) with petr ether–Et_2O mixtures gave 4.14 g of oily **10b**, R_f 0.35 (3CE).

3-[2-(1-Adamantyl)ethoxy]-1-propanol (12d) (Table V).—A soln of 12.6 g (0.07 mole) of 2-(1-adamantyl)ethanol[27] in 70 ml of anhyd pyridine was cooled to 0° and treated dropwise with 12.0 g (0.105 mole) of $MeSO_2Cl$. The mixt was stirred at 0° for 2 hr and at 25° for 4 hr, dild with 500 ml of Et_2O, and washed rapidly with cold H_2O, iced dil HCl, H_2O, cold 5% $NaHCO_3$, and H_2O. The dried (Na_2SO_4) ethereal soln was concd to 18 g (100%) of crude mesylate, R_f 0.44 (3CE), which was used without further purification.

NaH (11.39 g of a 60% mineral oil dispersion, 0.286 mole) in anhyd DMSO (200 ml) was heated at 65–70° for 1 hr under N_2 and cooled to 25° and a soln of propane-1,3-diol (21.7 g, 0.286 mole) in DMSO (25 ml) was added rapidly. The mixt was then stirred at 25° for 0.5 hr and a soln of 12.3 g (0.048 mole of 2-(1-adamantyl)ethanol-1-mesylate in 25 ml of DMSO was added at a rapid rate. The mixt was stirred an addnl 2 hr. Ice H_2O (1.5 l.) was used to quench the reaction and the product was extd into EtOAc, washed with brine, dried (Na_2SO_4), and concd to an orange oil. This was chromatographed on 540 g of Florisil, first with petr ether–Et_2O mixtures and then with Et_2O to give 6.37 g of colorless, oily **12d**, R_f 0.23 (3CE).

***O*-[3-(2-(1-Adamantyl)ethoxy)-1-propylphosphoryl]ethanolamine (13d) (Table VI).**—The procedure for this preparation was entirely similar to that for **6j** (method B). From 3.55 g (0.015 mole) of **4** and 4 ml of pyridine in $CHCl_3$, there was obtained one major P-positive component, R_f 0.58 (CMW); this was treated with 18 g of activated Zn in 40 ml of Et_2O and 20 ml of 90% AcOH. The usual work-up gave the crude product which was chromatogd on 800 g of 2:1 silica gel–Supercel with 4:1, v/v, $CHCl_3$–MeOH (4 l.), 3:1 $CHCl_3$–MeOH (6 l.), and 2:1 $CHCl_3$–MeOH (6 l.). The homogeneous fractions from the 3:1 and 2:1 eluates were crystd to give white **13d**, R_f 0.33 (CMW).

***O*-[3-(*cis*-9-Octadecenyloxy)-1-propylphosphoryl]ethanolamine (13b). Method C (Table VI).**—A soln of 10.0 g (0.031 mole) of 3-(*cis*-9-octadecenyloxy)-1-propanol (**12b**), 10.5 of the dicyclohexylammonium salt of *N*-β,β,β-trichloroethoxycarbonyl-2-aminoethylphosphoric acid (**14**),[7] 100 ml of anhyd pyridine, and 43 g of CCl_3CN[17] was heated on a steam bath for 4 hr under a positive head of N_2. The dark reaction mixt was cooled, dild with 400 ml of H_2O, and washed with Et_2O (6 × 100 ml). The aq layer was concd *in vacuo* to a brown syrup which was dissolved in 2:1, v/v, THF–H_2O (100 ml) and stirred with 30–40 g of Amberlite IR 120 (H^+) for 15 min. After the resin was filtered and the filtrate was concd *in vacuo*, the residue was dissolved in 100 ml of Et_2O and 50 ml of 90% AcOH and treated with 20 g of Zn and worked-up as in method B. The crude **13b** was a gum (8 g) which was chromatogd on 800 g of 2:1 silica gel–Supercel using 9:2, v/v, $CHCl_3$–MeOH. The homogenous cuts were crystd to give buff-colored **13b**, R_f 0.56 (CMW).

Bioassay Procedure.—In 1 ml of ethylene glycol was dissolved 5 mg of the potential inhibitor, then 1 ml of an aq soln containing 12 μmoles of hog renin substrate[28] was added, followed by 0.5 ml of 0.9% saline soln containing 1 unit of hog renin.[29] One to two drops of a 5% soln of diisopropylphosphorofluoridate (DFP) in *i*-PrOH was introduced and the mixt was adjusted to pH 6 with about a drop of 1 *M* NaH_2PO_4 soln. The total vol was about 2.5 ml giving an inhibitor concn of 2 mg/ml. This mixt was incubated at 37° for 1 hr, then placed in a boiling H_2O bath for 10 min, chilled in a solid CO_2–Me_2CO bath, and finally lyophilized. The residue was reconstituted by the addn of 1 ml of H_2O and the angiotensin I that was formed was measured by its pressor effect on the anesthetized rat prepn. This was similar to the procedure described by Pickens, *et al.*[18] Adult, male, Charles River rats, weighing 160–210 g, were used. Mean arterial blood pressure was measured from the carotid artery by means of a Sanborn transducer (Model 267 AC) and recorded on a multichannel Sanborn recorder (Model 964). Drug solns were given by means of a cannulated jugular or femoral vein. The prepn was allowed to equilibrate for 1 hr before proceeding with the expt. Then several injections of a standard dose of pure angiotensin II[30] were given to insure that the prepn was responding in a consistent manner. Two concns of the standard and 2 concns of the test soln were injected into each rat, and the resultant pressor response was plotted *vs.* log dose of soln (ml). The concn of angiotensin II produced was calcd from a standard curve. A minimum of 4 bioassays (4 rats) were used to evaluate each test soln. Control samples were worked-up as above but no potential inhibitor was added.

Biochemical Assay Procedure.—The potential renin inhibitors were dissolved in a small amount of Et_2O to which was added sufficient 0.05 *M* phosphate buffer (pH 7.4) made up in 0.1 *M* NaCl to give a concn of 3 mg/ml of test compd. This mixt was placed in an ice bath and dispersed with a Branson sonifer for about 5 min under N_2. One milliliter of this fine micelle suspension was added to a 50-ml round bottom centrifuge tube containing a mixt of 0.3 unit of hog renin[29] in 0.3 ml of H_2O, 0.1 ml of 0.003 *M* phenylmercuric acetate, 1 drop of DFP, and 1.1 ml of 0.05 *M* phosphate buffer (pH 7.4). During all these additions the tubes were kept in an ice bath. The mixts were incubated at 37° for 10 min in a New Brunswick metabolic shaker. Then 0.5 ml of phosphate buffer contg 10.2 nmoles of tetradecapeptide[31] renin substrate was added, and the incubation was continued for 10 min at 37°. The reaction was stopped by placing the vessels in a boiling H_2O bath for 10 min. Zero reaction time samples (blanks) were placed directly in the boiling H_2O bath after the 10-min preincubation and, after 5 min, 10.2 nmoles of renin substrate were added and then allowed to remain in the boiling H_2O bath for another 5 min.

The samples were cooled to 0°, 0.1 ml of a soln containing 1 *M* NaH_2PO_4 and 0.1 *M* NaCl (pH 4.1) was added followed by 0.3 ml of phosphate buffer containing 15 mg of converting enzyme.[28] The final pH was about 6.5. The tubes were incubated at 37° for 1 hr in the shaker. Ice-cold EtOH (13.6 ml) was added to stop the reaction, the tubes were placed in boiling H_2O for 4 min and then centrifuged. The supernatant was sepd, evapd under a stream of N_2, dissolved in 1 ml of distd H_2O, and placed on a column (130 × 10 mm) contg 1.55 g of Dowex 50W-X4 (H^+) (100–200 mesh). Each tube was rinsed (2X) with 1 ml each of H_2O and added to the column. The columns were eluted with 10 ml of 1:1, v/v, $CHCl_3$–MeOH, 50 ml of H_2O, 15 ml of 1 *N* HCl, 5 ml of 2 *N* HCl, and 50 ml of H_2O. Two 3-ml aliquots of 1 *N* NaOH were then added to the columns; the first 3 ml of effluent was discarded and the second 3-ml fraction was collected. Two milliliters of the final effluate was used for the fluorometric assay of His-Leu. (A complete elaboration of the biochemical assay used in this study will be reported by Green and Erickson in the near future.)

To 2 ml of effluate was added 0.1 ml of a soln of 0.1% orthophthaldialdehyde in abs EtOH, and the soln was then allowed to stand for 6 min. Then 0.7 ml of 3 *N* HCl was added and the sample was placed in a Turner 210 spectrophotofluorometer. The scan of emission intensity from 4500 to 6500 Å at an activation energy of 3696 Å gives a plot of fluorescence units *vs.* wavelength. The His-Leu concn was determined from a standard curve which had been corrected for extraneous fluorescence. This was obtained from the zero reaction time sample and was subtracted from the observed readings of the test samples. Control samples had no potential inhibitor added.

Enzymatic Preparation of 7. Phospholipase A Hydrolysis of *O*-[1,2-Diacyl-3-*sn*-glycerophosphoryl]ethanolamines (6).—Compds **6** (15–50 mg) were dissolved in 5 ml of Et_2O and added to a soln of 1.0 ml of 0.5 *M* tris(hydroxymethyl)aminomethane·HCl (pH 7.4), 1.0 ml of 0.0025 *M* $CaCl_2$, and 10 mg of lyophilized venom from *Crotalus adamanteus*.[32] The mixt was stirred overnight at 25° under N_2 and dild with 2.0 ml of abs EtOH and most of the Et_2O was evapd in a stream of N_2. Extn with heptane (3 × 5 ml) removed the fatty acids and the lyso derivative **7** was extd into $CHCl_3$ (2 × 10 ml), dried (Na_2SO_4), filtered, and concd in a stream of N_2. This material was weighed and used directly in the assays. The enzymatic hydrolyses of **6** were monitored by tlc in the system CMW: fatty acids, R_f 0.85–0.95; compds **6**, R_f 0.5–0.7; and compds **7**, R_f 0.3–0.35.

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(29) Nutritional Biochemicals Corporation, Cleveland, Ohio.
(30) Ciba Corporation, Summit, N. J.
(31) Schwarz/Mann, Orangeburg, N. Y.
(32) Pierce Chemical Company, Rockford, Ill.

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Potential Folic Acid Antagonists. 4. Synthesis and Dihydrofolate Reductase Inhibitory Activities of 2,4,6-Triamino-5-arylazopyrimidines

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A series of 49 2,4,6-triamino-5-arylazopyrimidines have been synthesized and examined for their inhibitory activity toward chicken liver dihydrofolate reductase. This activity was comparatively independent of the substituent character of the 5-aryl group; however, para substituents (almost without exception) reduced activity, meta substituents maintained activity, and small *o*-alkyl substituents increased activity. The most active compound in the series was found to be 2,4,6-triamino-5-*o*-ethylphenylazopyrimidine.

Previous studies in this laboratory[1-3] and elsewhere[4-7] have revealed that 5-arylazopyrimidines exhibit significant inhibitory activity towards folate–dihydrofolate reductase. In continuation of our studies of the structural requirements for this activity we report the synthesis and inhibitory activities of a series of 2,4,6-triamino-5-arylazopyrimidines.

Experimental Section[8]

Synthetic Procedure.—2,4,6-Triamino-5-arylazopyrimidines (Table I) were prepd by the general method described by Timmis and his coworkers.[4] The diazotized amine (0.1 mole) free from HNO_2 was added to a vigorously stirred soln of 2,4,6-triaminopyrimidine (0.1 mole) in H_2O (350 ml) at 0° with sufficient NaOAc to maintain the pH at 6–7. When the addn was complete, the mixt was stirred at 0–5° for 10 hr and then at 10° for 2–3 days. The product was collected and washed well with H_2O and air-dried: crude yields ranged from 70 to 100%. The compds were recrystd from *i*-PrOH which caused considerable losses but had the advantage of giving analytically pure specimens with the minimum of manipulation.

Enzyme Procedure.—Dihydrofolate reductase was partially purified from chicken liver according to Kaufman and Gardiner:[9] their procedure was followed to step IV, yielding a prepn with an increase in specific activity of 125- to 150-fold over that of the starting supernatant.

The standard assay mixt contained 5×10^{-5} *M* TPNH and 6×10^{-5} *M* dihydrofolate[10] in 50 m*M* phosphate buffer at pH 7.9. The velocity was detd spectrophotometrically at 340 n*M* using a cell of 1-cm path length. Inhibitors were added as aq solns if sol, or if insol in H_2O, in DMSO to give a final concn of 3.3% DMSO. When DMSO was employed the controls contained an equal amt of the solvent. Experiments with 10 water-sol compds selected at random showed that this concn of DMSO had little effect on the extent of inhibition produced. Initial velocities were detd over the first 2 min of the reaction in the presence of at least 6 inhibitor concns which were chosen to give a range of inhibition from 20 to 80%. From these data the $[I]/[S]_{0.5}$ values listed in Table I were determined. The $[I]/[S]$ ratios for 50% inhibition provide a convenient numerical manner for denoting changes in the affinity of an antagonist[1] with structure since when $[S] > 5K_m$, $[I]/[S]_{0.5} = K_i/K_m$.

Results and Discussion

It is quite apparent from the data of Table I that many of the substituents have but modest effects on the inhibitory activity of the parent compound, 2,4,6-triamino-5-arylazopyrimidine (**1**). In general, the introduction of para substituents (**4**,**7**,**12**,**15**,**17**,**21**,**23**, **26**,**28**,**31**,**34**) reduces activity relative to the parent compound: this effect is most marked with hydrocarbon substituents (**4**,**7**,**9**,**12**,**15**,**17**) although there is no obvious correlation with substituent size. In contrast, introduction of the more polar *p*-Cl, *p*-Br, *p*-I, *p*-MeO, and *p*-CO_2Et substituents produces a smaller reduction in activity; 2,4,6-triamino-5-*p*-iodophenylazopyrimidine (**26**) appears to be the sole example in which a para substituent increases activity above that of the parent unsubstituted compound (**1**).

Introduction of meta substituents (**3**,**6**,**14**,**18**,**20**,**22**, **25**,**27**,**30**,**33**,**35**) has only modest effects on the inhibitory activity. In general, activity is maintained or increased slightly and this is most marked with *m*-I (**25**) which shows a 4-fold increase. These effects of meta substituents appear to be relatively independent of substituent character.

The effect of nonpolar ortho substituents reaches a maximum at *o*-Et (**5**) which shows a 5.5-fold increase in activity: further increases in size of the substituent does not produce any further increase in activity. Other substituents (Cl, CF_3, MeO_2C, CF_3) do not increase activity and it is interesting that the iodo sub-

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