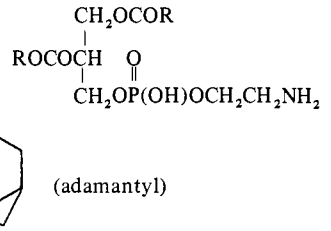
Potential Renin Inhibitors. 2. Ethanolamine and Ethylamine Derivatives of Phospholipids

Francis R. Pfeiffer,* Clara K. Miao, Suzanne C. Hoke, and Jerry A. Weisbach

Research and Development Division, Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101. *Received April 12, 1971*

Ethanolamine and ethylamine derivatives were prepared and evaluated as potential renin inhibitors in two *in vitro* assays. Compounds containing a 1-adamantyl moiety and an ethanolamine side chain were found to have maximal activity.

Several papers have described the isolation and assay of a phospholipid reported to be a natural precursor of a renin inhibitor.[1-6] A renin inhibitor may have utility as a mediator of the renin-angiotensin-aldosterone system in the treatment of certain hypertensive conditions.[7] Prompted by these findings we initiated a study to obtain stable, synthetic inhibitors, and have recently reported[8] on the renin inhibitory properties of a series of phosphatidylethanolamines and 2-desoxylysophosphatidylethanolamines. Two compounds, **1a** and **1b**, which contain 1-adamantyl and ethanolamine moieties, were found to be nearly as active as the inhibitor derived from natural sources. The synthesis of a number of analogs of these inhibitors was undertaken in order to define structural requirements and increase potency. This was approached *via* (1) altering the hydrophobic portion of the model compounds, (2) changing the oxidation state of P, and (3) substituting an ethylamine or choline group for the ethanolamine moiety.



1a, R = (adamantyl)

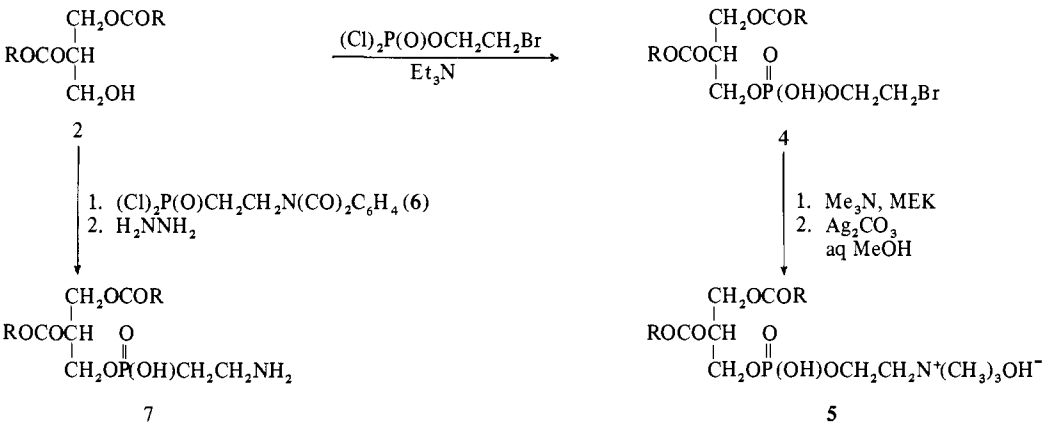
b, R = CH_2–adamantyl

Chemistry. 1,2-Bis(1-adamantoyl)-3-*sn*-phosphatidylcholine (**5**) was prepared in 2 steps from 1,2-bis(1-adamantoyl)-*sn*-glycerol (**2**)[8] (Scheme I). Phosphorylation of **2** with β-bromoethyl phosphorodichloridate (**3**) followed by displacement of the Br with Me_3N gave the quaternary methobromide salt which was converted to **5** with Ag_2CO_3.[9] The phosphono derivative of **1a**, 1,2-bis(1-adamantoyl)-*sn*-glycerol 2-aminoethyl phosphonate (**7**), was prepared by treatment of **2** with phthalimidoethylphosphonic acid dichloride (**6**)[10] and subsequent removal of the phthalimido group with hydrazine.

Compounds **13** and **18** are related to the 2-desoxylysophosphatidylethanolamines reported previously[8] which had renin inhibitory activity *in vitro*. The ether intermediates **10** and **11** required for the synthesis of **13** and **18** were obtained by treatment of an excess of the appropriate diol in DMSO and NaH with the mesylate **8** (at 25°) or the mesylate **9** (at 95°) (Scheme II). 6-[2-(1-Adamantyl)ethoxy]-hexan-1-ol (**10**) was phosphorylated with **12**[11] and the derived phosphate ester was treated with Zn–AcOH to remove the CCl_3CH_2OCO group to provide 6-[2-(1-adamantyl)-ethoxy]-1-hexyl 2-aminoethyl phosphate (**13**). The tosylate of **11** was heated in THF with sodium diethyl phosphite to afford diethyl 3-(1-adamantylmethyloxy)phosphonate (**14**). Alkaline hydrolysis of **14** gave the mono phosphonate ester **15** which was converted to the phosphonic acid **16** with hot HCl–AcOH. Esterification of **16** with *N-tert*-butyloxycarbonyl-2-aminoethanol (**17**) in the presence of CCl_3CN[12] in pyridine followed by acid hydrolysis of the $(CH_3)_3COCO$ group gave *O*-[3-(1-adamantylmethyloxy)propyl-1-phosphono]-2-aminoethanol (**18**).

Compds **21** and **24** illustrate other departures from the basic structure of the phosphatidylethanolamine molecule. 2-(1-Adamantyl)ethanol (**19**) was converted to the ethanolamine derivative **20** *via* reaction with **12**, and [2-(1-adamantyl)ethyl] 2-aminoethyl phosphate (**21**) was obtained

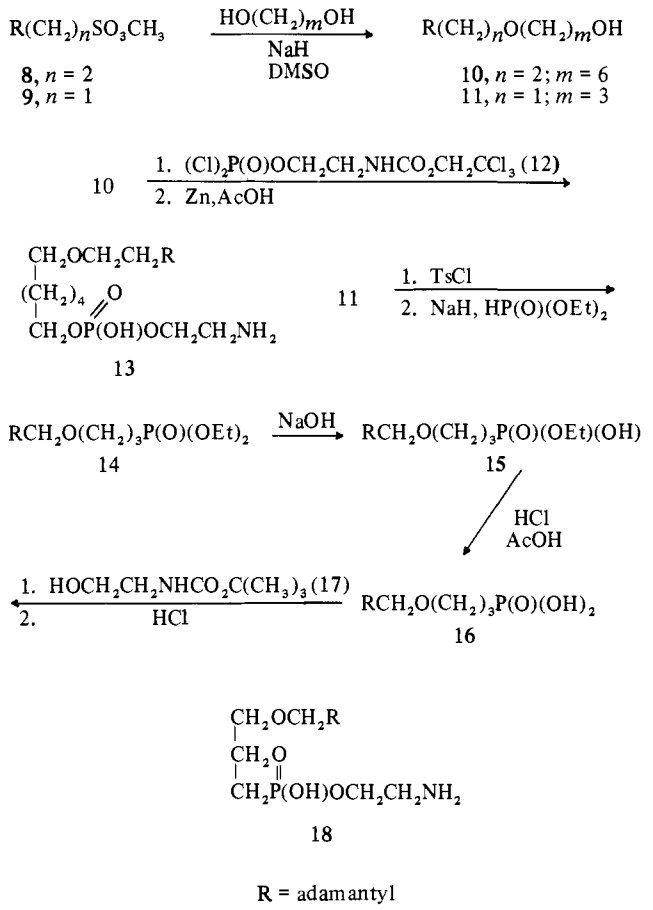
Scheme I



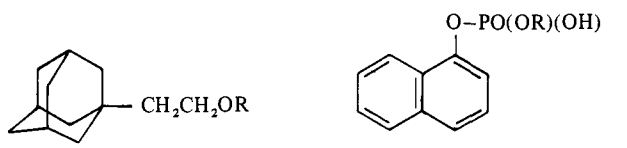
7 5

R = adamantyl

Scheme II



from **20** after Zn–AcOH treatment. 1-Naphthyl phosphate (**22**) and **17** gave **23** which was smoothly hydrolyzed with TFA to 1-naphthyl 2-aminoethyl phosphate (**24**).



19, R = H
20, R = $PO(OH)OCH_2$-$CH_2NHCO_2CH_2CCl_3$
21, R = $PO(OH)OCH_2CH_2NH_2$

22, R = H
23, R = $CH_2CH_2NHCO_2C(CH_3)_3$
24, R = $CH_2CH_2NH_2$

Biological Evaluation. The ability of these compounds to inhibit the renin-catalyzed conversion of renin substrate to angiotensin I was examined in 2 *in vitro* test systems.[8] The biological data may be summarized as follows (Table I). Compd **21** showed interesting activity in the biochemical assay and was the most potent inhibitor of this series. Only **1b** (68% inhibition at $1.76 \times 10^{-3} M$ in the biochemical assay) was more active than **21**. Somewhat surprisingly, the naphthyl derivative **24** was essentially inactive and differed from **21** mainly at the hydrophobic portion of the molecule. A literature report[1] suggested that phospholipids of the phosphatidylcholine type were not active as renin inhibitors in a bioassay procedure. Compd **5**, a phosphatidylcholine having two 1-adamantoyl groups, exhibited good activity in the bioassay. A change in oxidation from phosphate to phosphonate of **7** and **18** showed decreased activity when compared to **1a** and some 2-desoxylysophosphatidylethanolamines reported previously. In summary, renin inhibitory activity was found in compounds structurally fairly distant from the active phosphatidylethanolamines isolated from tissue. The most active compounds contained an ethanolamine group and, preferably, an adamantyl moiety at the hydrophobic end.

Table I. Biological Evaluation of Potential Renin Inhibitors[a]

	Biochemical assay		Bioassay	
Compound	Concn, $\times 10^{-3} M$	Inhibition, %[b]	Concn, $\times 10^{-3} M$	Inhibition, %[b]
5			3.0	52
7	1.9	11	3.8	37
13	1.25	19		
	2.5	40		
18	1.5	16		
	2.9	42		
21	1.7	51		
24	1.9	0		
	4.0	<5		

[a] The detailed descriptions of the biochemical assay and the bioassay were given previously (ref 8). [b] Per cent change when compared to controls.

Experimental Section

The compds were routinely checked by ir; mps were detd in a Thomas-Hoover app and are cor; optical rotations were taken on a Perkin-Elmer 141 polarimeter. Tlc was carried out on 0.25 mm Analtec silica gel GF plates; solvent system A is $CHCl_3$–MeOH–H_2O (65:25:4). The compds were detected by spraying with 40% H_2SO_4 and heating, with ninhydrin, and with a reagent for P.[13] Where analyses are indicated only by symbols of the elements, analytical results obtained were within ±0.4% of the theoretical values, and were obtd by the Analytical and Physical Chemistry Section of Smith Kline & French Laboratories. Petr ether used had bp 30–60°. Mallinckrodt silica gel (100 mesh) mixed with 0.5 part of Supercel was used for column chromatog except where indicated.

1,2-Bis(1-adamantoyl)-3-*sn*-phosphatidylcholine (5). A soln of 25.26 g (0.104 mole) of β-bromoethyl phosphorodichloridate (**3**)[14] in 15 ml of anhyd $CHCl_3$ (distd from P_2O_5) was added dropwise at 0° to a soln of 14.5 g (0.0348 mole) of **2**[8] in 21 ml of Et_3N and 35 ml of anhyd $CHCl_3$. The mixt was kept at 5° overnight, then stirred at 25° for 2 hr. The $CHCl_3$ was evapd (<30°), 150 ml of 0.1 *N* KCl and 60 ml of Et_2O–MeOH (10:1) were added, and the mixt was stirred at 0° for 1 hr. Et_2O (800 ml) was added and dil HCl was added to pH 2. The layers were sepd, and the Et_2O was washed well with H_2O, dried (Na_2SO_4), and concd to 21 g of syrupy **4**. Tlc ($CHCl_3$–MeOH, 7:1) showed the major product R_f 0.21. The crude **4** was dissolved in 200 ml of anhyd MEK contg 20 g of Me_3N, and held at 50° for 18 hr under a positive head of N_2.

The suspension was concd to about 75 ml *in vacuo*, and the solid was filtered and washed with cold MEK to give 13.5 g of dried methobromide salt with R_f 0.58 (system A). This salt was dissolved in 400 ml of MeOH–H_2O (9:1), 19 g of Ag_2CO_3 was added, and the mixt was stirred vigorously for 3 hr, filtered, and concd (<40°). The residue was dild with 100 ml of $CHCl_3$–MeOH–H_2O (5:4:1) and percolated through a column packed with 100 g each of Amberlite IR 45 and IR 50. An addnl 300 ml of the same solvent was used to elute the column. The column washings were concd, and the residue was placed on a column of 900 g of the silica gel mixture. Elution with system A and crystn of the homogeneous cuts from $CHCl_3$–MEK with a trace of abs EtOH gave 5.5 g of white **5** (26%): mp 270–272°; $[\alpha]^{25}D$ + 7.2° (*c* 1.0, $CHCl_3$); R_f 0.46 (system A). *Anal.* ($C_{30}H_{50}NO_9P$ H_2O) C, H, N, P, H_2O.

1,2-Bis(1-adamantoyl)-*sn*-glycerol 2-Aminoethyl Phosphonate (7). A soln of 2.0 g (0.0048 mole) of **2**, 1.75 ml of anhyd pyridine, and 25 ml of anhyd $CHCl_3$ was cooled to 0° and treated dropwise with a soln of 1.35 g (0.005 mole) of phthalimidoethylphosphonic acid dichloride (**6**)[10] in 30 ml of anhyd $CHCl_3$. The addn was complete in 30 min, then the soln was stirred at 25° for 18 hr, dild with 200 ml of Et_2O, and washed with dil HCl and H_2O. The dried yellowish residue (3.7 g) was homogeneous with an R_f 0.89 (system A). The crude phthalimido derivative was stirred with 95% EtOH (50 ml)

and 50% H_2NNH_2 (1.75 ml) at 25° for 18 hr. A voluminous ppt sepd. The solvents were evapd, the residue was stirred with 75 ml of $CHCl_3$, and the phthalhydrazide was filtered. The filtrate was concd (2.5 g) and chromatogd on 250 g of the silica gel mixt starting with $CHCl_3$ and then with an increasing MeOH gradient. Most of the homogeneous product was eluted with 6:1 $CHCl_3$–MeOH. Crystn from $CHCl_3$–Et_2O gave 0.89 g (36%) of buff 7: mp 207–209°; $[\alpha]^{25}D$ + 6.2° (*c* 0.7, $CHCl_3$); R_f 0.76 (system A). *Anal.* ($C_{27}H_{42}NO_7P \cdot H_2O$) C, H, N, H_2O.

6-[2-(1-Adamantyl)ethoxy]-1-hexanol (10). A mixt of NaH (0.42 mole) in DMSO (300 ml) was heated to 65° for 1 hr (N_2) and cooled to 25°, and hexane-1,6-diol (49.6 g, 0.42 mole) in DMSO (100 ml) was added. The mixt was stirred for 30 min, a soln of 2-(1-adamantyl)ethyl mesylate (**8**)[8] (18 g, 0.07 mole) in DMSO (50 ml) was added, and the mixt was stirred at 25° for 7 hr. H_2O (3 l.) was added, the product was extd into C_6H_6–petr ether, and the exts were washed well with H_2O. The dried, oily residue (23.3 g) was chromatogd on 700 g of Florisil using a petr ether–Et_2O gradient to give colorless, oily 10 (9.5 g, 49%). *Anal.* ($C_{18}H_{32}O_2$) C, H.

3-(1-Adamantylmethyloxy)-1-propanol (11). The procedure for 10 was used except the mixt was stirred at 95° for 48 hr. From 0.3 mole of NaH, 0.3 mole of propane-1,3-diol, and 0.05 mole of 1-adamantylmethyl mesylate (9) (mp 75–76.5°, prepd from 1-adamantylcarboxylic acid by the method used for **8**) 3.8 g (34%) of the colorless, oily 11 was obtd after chromatog on Florisil. *Anal.* ($C_{14}H_{24}O_2$) C, H.

6-[2-(1-Adamantyl)ethoxy]-1-hexyl 2-Aminoethyl Phosphate (13). A soln of 3.1 g (0.011 mole) of 10, 4.5 ml of anhyd pyridine, and 30 ml of anhyd $CHCl_3$ was added dropwise at 0° to a soln of 5.93 g (0.0168 mole) of *N*-(β,β,β-trichloroethoxycarbonyl)-2-aminoethyl phosphorodichloridate (12),[11] and then the soln was stirred at 25° for 18 hr. The work-up and removal of the protecting group was entirely similar to the method described previously,[8] and gave 5.2 g of crude 13. This material showed one major spot on tlc which was ninhydrin and P positive. Chromatog on 500 g of the silica gel mixt with 20–30% MeOH in $CHCl_3$ followed by crystn from MeOH–MeCN gave 1.2 g (27%) of 13: mp 229–231°; R_f 0.39 (system A). *Anal.* ($C_{20}H_{38}NO_5P \cdot 0.5H_2O$) C, H, N, H_2O.

Monocyclohexylammonium Salt of 3-(1-Adamantylmethyloxy)-propyl-1-phosphonic Acid (16). A suspension of 3.67 g (0.09 mole of a 60% mineral oil dispersion) of NaH in 100 ml of anhyd THF (from 5A and 13X Linde Molecular Sieves) was dissolved by the portionwise addn at reflux of 20 ml of diethyl phosphite. A soln of 5.06 g (0.0134 mole) of crude 3-(1-adamantylmethyloxy)-1-propyl tosylate [from 3.0 g of 11, 5.1 g of TsCl, and 30 ml of pyridine at 25° for 3 hr; R_f 0.65 with cyclohexane–EtOAc (3:1)] was added and refluxing was contd for 18 hr. The THF was evapd, the residue was taken up in aq Et_2O, and the Et_2O layer was washed with H_2O. The dried, crude diethyl 3-(1-adamantylmethyloxy)-1-propylphosphonate (14) (11.6 g) was refluxed for 3 hr with EtOH (40 ml) and 20% NaOH (40 ml), concd, dild with H_2O, and extd with Et_2O. The aq phase was acidified, extd with EtOAc–$CHCl_3$ (4:1), and dried to give 3.1 g (82%) of oily, orange ethyl 3-(1-adamantylmethyloxy)-1-propyl phosphonate (**15**). A portion was converted in Me_2CO to the cyclohexylammonium salt and crystd from abs EtOH–Me_2CO–1% cyclohexylamine, mp 138–140°. *Anal.* ($C_{16}H_{29}O_4P \cdot C_6H_{13}N$) C, H, N.

The oily **15** (1.3 g) was refluxed for 18 hr with AcOH (5 ml) and concd HCl (2 ml) and then concd *in vacuo*, and the residue extd with Et_2O which was washed well with H_2O. The dried oily **16** was dissolved in Me_2CO, basified with cyclohexylamine, cooled, and filtered to give 1.1 g of the monocyclohexylammonium salt of **16**. Recrystn from abs EtOH–Me_2CO–1% cyclohexylamine afforded mp 220–222°. *Anal.* ($C_{14}H_{25}O_4P \cdot C_6H_{13}N$) C, H, N.

***O*-[3-(1-Adamantylmethyloxy)propyl-1-phosphono]-2-aminoethanol (18).** A soln of 0.87 g (0.0022 mole) of the monocyclohexylammonium salt of **16**, 1.09 g (0.0067 mole) of *tert*-butyloxycarbonylaminoethanol (17),[15] 11.3 ml of anhyd pyridine, and 3.2 g (0.022 mole) of CCl_3CN[12] was stirred at 50° for 18 hr, cooled, dild with 50 ml of H_2O, and extd with Et_2O (6×). The aq layer was acidified with HCl, extd with Et_2O, washed with brine, dried, and concd to a syrup which was azeotroped with anhyd C_6H_6. The *tert*-BuO derivative was dissolved in anhyd Et_2O and treated with a stream of dry HCl at 0° for 2 hr. After evapn the residue was dissolved in 75 ml of Et_2O–ETOH–H_2O (4:2:1) and percolated through 30 g of Amberlite IR 45 (OH^-) with an addl 225 ml of solvent. The eluate was concd and azeotroped several times with abs ETOH to afford nearly homogeneous **18**. Crystn first from $CHCl_3$–MeCN and then MeOH–CH_3CN gave 0.245 g (34%) of 18: mp 243–245°; R_f 0.48 (system A). *Anal.* ($C_{16}H_{30}NO_4P$) C, H, N.

[2-(1-Adamantyl)ethyl] 2-Aminoethyl Phosphate (21). The procedure used for the prepn of 13 was followed. From 4.23 g (0.012 mole) of 12 and 1.8 g (0.01 mole) of 2-(1-adamantyl)-ethanol (**19**), 5.23 g of crude **20** was obtd. This was treated with 10 g of Zn dust in 20 ml of Et_2O and 15 ml of 90% AcOH. The usual work-up gave 3.5 g of product. Tlc (system A) showed one major spot (R_f 0.52) which was ninhydrin and P positive and a minor component which was ninhydrin negative and P positive. Several fractional crystns from $CHCl_3$–MeCN gave buff crysts, mp 243–245°. *Anal.* ($C_{14}H_{26}NO_4P \cdot 0.5H_2O$) C, H, N.

Cyclohexylammonium Salt of 1-Naphthyl *N*-*tert*-Butyloxycarbonyl-2-aminoethyl Phosphate (23). A soln of 10.0 g (0.047 mole) of 1-naphthyl phosphate (22),† 21.2 g (0.131 mole) of **17**, 67.8 ml (0.47 mole) of CCl_3CN,[12] and 100 ml of anhyd pyridine was stirred on the steam bath for 4 hr. The soln was concd to one-third of the original vol, dild with 120 ml of H_2O, and extd with Et_2O (4 × 150 ml). The Et_2O exts were back washed with H_2O (2 × 100 ml), and the combined aq layers dild with 8 ml of cyclohexylamine and evapd to a tan solid. The moist solid was filtered with the aid of Et_2O and recrystd from 100 ml of DMF which contd 2 ml of cyclohexylamine. The white solid was washed well with Et_2O to provide 12.4 g (57%) of the white cyclohexylammonium salt of **23**, mp 173–175°. *Anal.* ($C_{17}H_{22}N_6P \cdot C_6H_{13}N$) C, H, N.

1-Naphthyl 2-Aminoethyl Phosphate (24). A soln of 10.0 g of 23 in 100 ml of MeOH was stirred with 35 g of wet Amberlite IR 120 (H^+) for 30 min and filtered, and the residue was azeotroped with dry C_6H_6 (4×). This reddish free base was dissolved in 10 ml of F_3CCO_2H and allowed to stand for 30 min at 25°. Then 150 ml of C_6H_6 was added, and the soln was evapd. The cryst residue was triturated with Me_2CO to give 5.5 g (96%) of 24, mp 252–254°. A recrystn from Me_2CO–H_2O (1:1) afforded 3.35 g of white solid, mp 265–267°. Addnl product was obtd by dilg the filtrate with CH_3CN: R_f 0.43 (system A). *Anal.* ($C_{12}H_{14}NO_4P$) C, H, N.

Acknowledgments. The authors are grateful to Miss Margaret Carroll and staff for elemental analyses, Mr. Walter Hamil and staff for optical rotations, Miss Josephine Pasternak and Miss Roberta Carraine for carrying out the bioassays, and Mr. Robert Erickson and Mrs. Charlotte Burton for testing the compounds in the biochemical assay.

References

(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. H. Osmond, L. A. Lewis, R. R. Smeby, and F. M. Bumpus, *ibid.*, **73**, 809 (1969).
(4) D. Ostrovsky, S. Sen, R. R. Smeby, and F. M. Bumpus, *Circ. Res.*, **21**, 497 (1967).
(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) K. J. Catt, M. D. Cain, J. P. Coghlan, P. Z. Zimmet, E. Cran, and J. B. Best, *ibid.*, **26/27**, *Suppl. II*, II-177 (1970).
(8) F. R. Pfeiffer, S. C. Hoke, C. K. Miao, R. E. Tedeschi, J. Pasternak, R. Hahn, R. W. Erickson, H. W. Levin, C. A. Burton, and J. A. Weisbach, *J. Med. Chem.*, **14**, 493 (1971).
(9) H. Eibl and O. Westphal, *Justus Liebigs Ann. Chem.*, **709**, 244 (1967), and references cited therein.
(10) A. F. Rosenthal, and M. Pousada, *Recl. Trav. Chim. Pays-Bas*, **84**, 833 (1965).
(11) F. R. Pfeiffer, S. R. Cohen, and J. A. Weisbach, *J. Org. Chem.*, **34**, 2795 (1969).
(12) F. Cramer, W. Rittersdorf, and W. Böhn, *Justus Liebigs Ann. Chem.*, **654**, 180 (1962).
(13) J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, **5**, 126 (1964).
(14) R. Hirt and R. Berchtold, *Pharm. Acta Helv.*, **33**, 349 (1958); *Chem. Abstr.*, **53**, 9283 (1959).
(15) F. J. M. Daemen, G. H. de Haas, and L. L. M. van Deenen, *Recl. Trav. Chim. Pays-Bas*, **82**, 487 (1963).

†Aldrich Chemical Co.