Four different commercial benzalkonium chlorides were analyzed after Hofmann degradation. Qualitatively similar chromatograms were obtained (Fig. 2), but analysis of peak areas showed considerable variation in the alkyl ratio distribution (Table III). This study was expanded to evaluate possible lot to lot variations in a commercial product used for an extended time and samples of benzalkonium chloride (product *D*) as received over a period of 2 years were analyzed for their alkyl distribution. Constant alkyl ratios were obtained (Table IV). Two lots of product *A* were also analyzed with similar good agreement for alkyl distribution between lots (Table V).

Impurities in Benzalkonium Chlorides—In addition to the expected alkenes, samples of benzalkonium chlorides showed the presence of impurities of alkanols, alkanes, and benzylmethylamine. Many of these impurities have been previously characterized (2) and can be identified in chromatograms by their retention data. Considerable variation in the amounts of the impurities between different benzalkonium chlorides were noted. An analysis of the peak areas of the major impurities in the chromatograms from Fig. 2 is shown in Table VI. The volatile impurities were a significant contribution to the content of the products and were from 30–53%. No evaluation of nonvolatile impurities was made.

Quantitation—The use of the modified Hofmann degradation approach for quantitative results is based on the formation of benzyldimethylamine by any of the quaternaries present, irrespective of the alkyl substituents. The ratios of the alkenes found allows an assignment of the amount of the particular quaternary. Quantitation was possible to a lower limit of 0.05%.

Comparison of Catalytic Hydrogenation and Hofmann Degradation—The official USP method for the determination of alkyl distribution in benzalkonium chloride requires specialized hydrogenation equipment in addition to a temperature programmed gas chromatograph (1). In the chemical work-up after hydrogenation and before analysis, many of the impurities possibly present in the original compounds or created with hydrogenation are eliminated by the USP procedure. The ratio of the amines resulting from the hydrogenation of the benzalkonium chloride are obtained from the chromatograms. A comparison of the USP procedure with the modified Hofmann degradation was made for two lots of product *A* (Table V) and for a USP reference standard (Table VII). The results obtained by both methods showed that the differences between methods were within the limits of variation for either method on a single sample.

REFERENCES

(1) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 66.
(2) Jennings, E. C., Jr., and Mitchner, H., *J. Pharm. Sci.*, **56**, 1590(1967).
(3) Warrington, H. P., Jr., *Anal. Chem.*, **33**, 1898(1961).
(4) Southworth, B. C., *ibid.*, **28**, 1611(1956).

N-Aralkyl-*N*-methylaminoethyl Carbanilates as Hypocholesteremic Agents

By WILLIAM J. ROST, BLAINE M. SUTTON, BENJAMIN BLANK, FRANCIS R. PFEIFFER, WILLIAM L. HOLMES, NICHOLAS W. DITULLIO, and EDWIN B. INGRAM

A group of aralkylaminoethyl esters and ureas of substituted carbanilic acids was prepared and studied for hypocholesteremic activity in eucholesteremic mice. Biological results indicated that modification of either terminal aromatic ring altered the activity of the resultant compound. The *meta* and *para* methyl substituted carbanilates of aralkylmethylaminoethanol consistently exhibited the most desirable effect. In the dicarbanilate series of aralkyl or aryl substituted iminodiethanols, no appreciable activity was seen.

A previous publication (1) reported on the preparation of a series of *N*-aralkyl-*N*-methylaminoethyl carbanilates and their local anesthetic activity. Since that report, additional biological testing revealed that certain members of the series possessed hypocholesteremic activity in experimental animals. The novelty of this activity as well as the potential value of a safe effective agent exhibiting these properties prompted the study of this type of compound in greater detail. This paper describes preparation of additional examples in this class of compounds, their effect on cholesterol metabolism in the mouse, and chemical structure-biological function relationships in the series.

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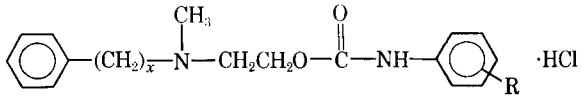
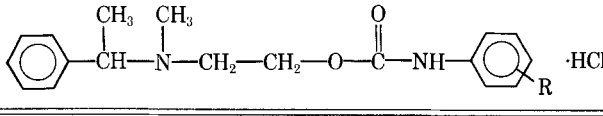
TABLE I—*N*-ARALKYL-*N*-METHYLAMINOETHYL CARBANILATE HYDROCHLORIDES



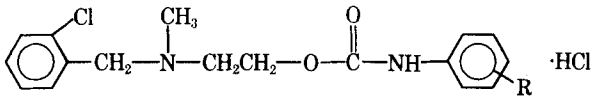
No.	x	R	M.p., °C.	Formula	Anal. Calcd.	Anal. Found
3	1	*o*-Cl	137–139	$C_{17}H_{20}Cl_2N_2O_2$	C, 57.47 H, 5.67 N, 7.89	57.60 5.71 7.83
6	1	*o*-CH_3	143–145	$C_{18}H_{23}ClN_2O_2$	C, 64.56 H, 6.92 N, 8.37	64.77 6.96 8.28
8	1	*o*-CH_3O	114–116	$C_{18}H_{23}ClN_2O_3$	C, 61.82 H, 6.61 N, 7.99	61.80 6.58 7.99
11	2	*m*-Cl	194–195	$C_{18}H_{22}Cl_2N_2O_2$	C, 58.54 H, 6.00 N, 7.59	58.47 6.15 7.53
12	2	*o*-Cl	165–166	$C_{18}H_{22}Cl_2N_2O_2$	C, 58.54 H, 6.00 N, 7.59	58.79 6.05 7.26
14	2	*m*-CH_3	185–186	$C_{19}H_{25}ClN_2O_2$	C, 65.41 H, 7.22 N, 8.03	65.54 7.18 7.99
15	2	*o*-CH_3	168–170	$C_{19}H_{25}ClN_2O_2$	C, 65.41 H, 7.22 N, 8.03	65.53 7.26 8.22
17	2	*o*-CH_3O	117–119	$C_{19}H_{25}ClN_2O_3$	C, 62.54 H, 6.91 N, 7.68	62.28 6.86 7.83
21	3	*o*-Cl	113–115	$C_{19}H_{24}Cl_2N_2O_2$	C, 59.53 H, 6.31 N, 7.31	59.49 6.27 7.22
24	3	*o*-CH_3	132–134	$C_{20}H_{27}ClN_2O_2$	C, 66.19 H, 7.50 N, 7.72	66.33 7.54 7.69
25	3	*o*-CH_3O	136–138	$C_{20}H_{27}ClN_2O_3$	C, 63.40 H, 7.18 N, 7.39	63.67 7.17 7.45
28	3	*p*-NO_2	177–179	$C_{19}H_{24}ClN_3O_4$	C, 57.94 H, 6.14 N, 10.67	57.63 6.09 10.64
29	3	*p*-NH_2	220–222	$C_{19}H_{27}Cl_2N_3O_2$	C, 57.00 H, 6.80 N, 10.50	57.19 6.88 10.41

TABLE II—*N*-α-METHYLBENZYL-*N*-METHYLAMINOETHYL CARBANILATE HYDROCHLORIDES



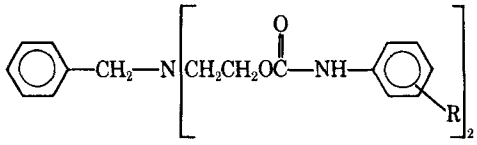
No.	R	M.p., °C.	Formula	Anal. Calcd.	Anal. Found
30	*p*-CH_3	195–197	$C_{19}H_{25}ClN_2O_2$	C, 65.41 H, 7.22 N, 8.03	65.34 7.07 8.03
31	*m*-CH_3	125–127	$C_{19}H_{25}ClN_2O_2$	C, 65.41 H, 7.22 N, 8.03	65.57 7.19 8.12

TABLE III—*N*-*o*-CHLOROBENZYL-*N*-METHYLAMINOETHYL CARBANILATE HYDROCHLORIDES



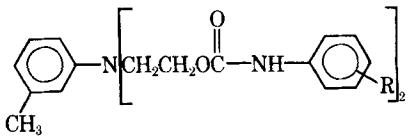
No.	R	M.p., °C.	Formula	Anal. Calcd.	Anal. Found
32	*p*-Cl	208–210	$C_{17}H_{19}Cl_3N_2O_2$	C, 52.37 H, 4.91	52.41 4.75
33	*m*-Cl	192–193	$C_{17}H_{19}Cl_3N_2O_2$	C, 52.37 H, 4.91	52.26 4.92
34	*p*-CH_3	187–189	$C_{18}H_{22}Cl_2N_2O_2$	C, 58.54 H, 6.00	58.64 6.00
35	*m*-CH_3	203–205	$C_{18}H_{22}Cl_2N_2O_2$	C, 58.54 H, 6.00	58.56 6.00
36	*p*-CH_3O	184–186	$C_{18}H_{22}Cl_2N_2O_3$	C, 56.11 H, 5.75	56.17 5.83

TABLE IV—*N*-BENZYLIMINODIETHYL DICARBANILATES



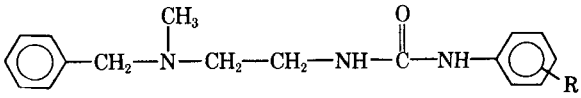
No.	R	M.p., °C.	Formula	Anal. Calcd.	Anal. Found
37	*p*-Cl	115	$C_{25}H_{25}Cl_2N_3O_4$	C, ... H, ...	... ...
38	*m*-Cl	106	$C_{25}H_{25}Cl_2N_3O_4$	C, 59.76 H, 5.01	60.10 5.10
39	*p*-CH_3	122–123	$C_{27}H_{31}N_3O_4$	C, 70.25 H, 6.76	70.34 6.80
40	*m*-CH_3	74–75	$C_{27}H_{31}N_3O_4$	C, 70.25 H, 6.76	70.29 6.69
41	*p*-CH_3O	100	$C_{27}H_{31}N_3O_6$	C, 65.70 H, 6.33	65.81 6.27

TABLE V—*N*-*m*-TOLYLIMINODIETHYL DICARBANILATES



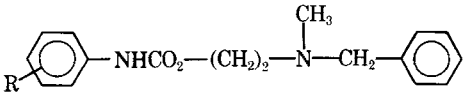
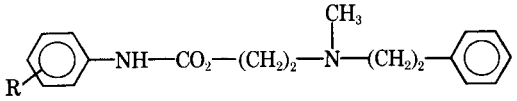
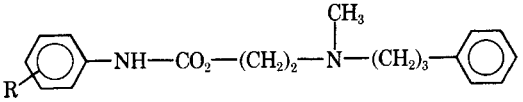
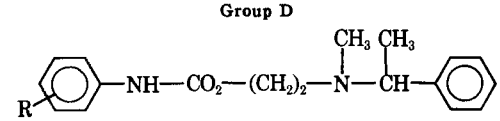
No.	R	M.p., °C.	Formula	Anal. Calcd.	Anal. Found
42	*p*-Cl	136–137	$C_{25}H_{25}Cl_2N_3O_4$	C, 59.76 H, 5.01	59.88 5.07
43	*m*-Cl	126–127	$C_{25}H_{25}Cl_2N_3O_4$	C, 59.76 H, 5.01	59.89 5.13
44	*p*-CH_3	151	$C_{27}H_{31}N_3O_4$	C, 70.25 H, 6.76	70.41 6.80
45	*m*-CH_3	125–126	$C_{27}H_{31}N_3O_4$	C, 70.25 H, 6.76	70.25 6.75
46	*p*-CH_3O	131	$C_{27}H_{31}N_3O_6$	C, 65.70 H, 6.33	65.84 6.46

TABLE VI—*N*-BENZYL-*N*-METHYLAMINOETHYL-*N*-ARYLUREAS



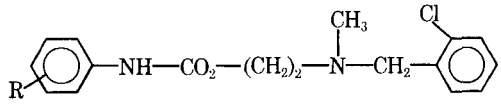
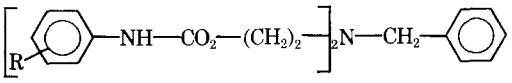
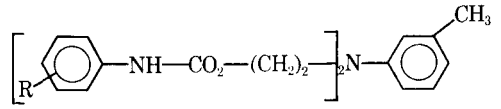
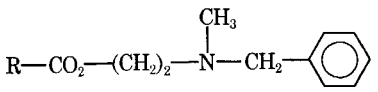
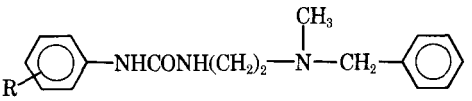
No.	R	M.p., °C.	Formula	Anal. Calcd.	Anal. Found
50	*p*-Cl	118–120	$C_{17}H_{20}ClN_3O$	C, 64.25 H, 6.34	64.39 6.34
51	*m*-Cl	117–119	$C_{17}H_{20}ClN_3O$	C, 64.25 H, 6.34	64.09 6.19
52	*p*-CH_3	116–118	$C_{18}H_{23}N_3O$	C, 72.69 H, 7.80	72.64 7.80
53	*m*-CH_3	127–129	$C_{18}H_{23}N_3O$	C, 72.69 H, 7.80	72.51 7.84
54	*p*-CH_3O	97–98	$C_{18}H_{23}N_3O_2$	C, 68.98 H, 7.40	68.78 7.34

TABLE VII—RESULTS OF BIOLOGICAL ASSAY OF *N*-ARALKYL-*N*-METHYLAMINOETHYL CARBANILATES

No.	R	Plasma Cholesterol, % Change from Control	Liver Cholesterol, % Change from Control	No.	R	Plasma Cholesterol, % Change from Control	Liver Cholesterol, % Change from Control
		Group A					
							
1	*p*-Cl	−13.5	−0.8	*6*	*o*-CH_3	−11.5	+18.0
2	*m*-Cl	−11.3	−0.5	*7*	*p*-CH_3O	−1.4	−13.4
3	*o*-Cl	−7.7	+0.9	*8*	*o*-CH_3O	−20.5	+13.9
4	*p*-CH_3	−27.9	−12.1	*9*	H	−19.2	+18.6
5	*m*-CH_3	−13.9	−18.2				
		Group B					
							
10	*p*-Cl	−21.6	−19.3	*15*	*o*-CH_3	−6.2	+3.7
11	*m*-Cl	−11.5	+10.5	*16*	*p*-CH_3O	−6.3	−15.5
12	*o*-Cl	−26.9	+17.4	*17*	*o*-CH_3O	−20.5	+11.7
13	*p*-CH_3	−11.3	−25.4	*18*	H	+6.7	−13.3
14	*m*-CH_3	−20.4	−16.0				
		Group C					
							
19	*p*-Cl	−14.3	+7.7	*25*	*p*-CH_3O	−7.6	−18.2
20	*m*-Cl	−5.3	−26.4	*26*	*o*-CH_3O	−1.3	+29.6
21	*o*-Cl	+10.0	+18.8	*27*	H	−7.0	−8.4
22	*p*-CH_3	−29.0	−16.9	*28*	*p*-NO_2	−12.7	+4.7
23	*m*-CH_3	−20.9	−25.2	*29*	*p*-NH_2	0.0	+10.3
24	*o*-CH_3	−9.7	−31.3				
		Group D					
							
30	*p*-CH_3	−1.4	+16.9	*31*	*m*-CH_3	−1.3	+18.2

(*Continued on next page.*)

TABLE VII—(*Continued.*)

No.	R	Plasma Cholesterol, % Change from Control	Liver Cholesterol, % Change from Control	No.	R	Plasma Cholesterol, % Change from Control	Liver Cholesterol, % Change from Control
		Group E					
							
32	*p*-Cl	+4.7	+10.6	*35*	*m*-CH_3	+18.0	+19.3
33	*m*-Cl	+25.3	+70.8	*36*	*p*-CH_3O	+3.4	+16.8
34	*p*-CH_3	+4.0	+15.8				
		Group F					
							
37	*p*-Cl	+14.9	+14.8	*40*	*m*-CH_3	−3.8	+27.2
38	*m*-Cl	+14.2	+20.8	*41*	*p*-CH_3O	−4.5	+0.2
39	*p*-CH_3	+18.0	+18.1				
		Group G					
							
42	*p*-Cl	+17.8	−7.1	*45*	*m*-CH_3	+10.9	+6.1
43	*m*-Cl	−5.8	−7.1	*46*	*p*-CH_3O	−1.5	−3.8
44	*p*-CH_3	+1.5	+8.0				
		Group H					
							
47	$(CH_3)_2N$-	−1.6	−21.7	*48*	C_4H_9-NH-	−21.6	−15.7
		Group I					
							
49	*p*-Cl	+4.0	+11.9	*52*	*m*-CH_3	+23.1	−9.0
50	*m*-Cl	+19.5	+44.6	*53*	*p*-CH_3O	−20.1	−11.6
51	*p*-CH_3	−0.6	+8.0				

EXPERIMENTAL

Chemical

Preparation of N-Aralkyl-N-methylaminoethyl Carbanilate Hydrochlorides—The compounds listed in Tables I, II, and III were prepared from the appropriate alcohol and substituted phenyl isocyanate in the manner previously described by Barnes and Rost (1).

Preparation of N-Benzyliminodiethyl Dicarbanilates—The compounds listed in Table IV were prepared from *N*-benzyliminodiethanolamine and substituted phenylisocyanate in the same manner as that described for *N*-benzyliminodiethyl di-*p*-chlorophenylcarbanilate by McKay and Hatton (2).

Preparation of N-m-Tolyliminodiethyl Dicarbanilates—The compounds in Table V were prepared from *m*-tolyliminodiethanol and the appropriate substituted phenyl isocyanate in the same manner as the *N*-benzyliminodiethyl dicarbanilates previously described.

Preparation of N_1-(N-Benzyl-N-methylaminoethyl)-N_3-p-chlorophenylurea—The compound was prepared by dissolving 3.25 Gm. (0.02 mole) of *N*-benzyl-*N*-methylaminoethylamine in 10 ml. of toluene and adding 3.2 Gm. (0.022 mole) of *p*-chlorophenylisocyanate which was dissolved in 10 ml. of toluene. After refluxing 1 hr., the mixture was permitted to stand overnight. The crystals which formed were filtered off and recrystallized from acetone. The 3.4 Gm. yield (53%) melted at 118–120°.

The compounds listed in Table VI were prepared in the same manner as that described for N_1-(*N*-benzyl-*N*-methylaminoethyl)-N_3-*p*-chlorophenylurea.

Preparation of N-Benzyl-N-methylaminoethyl-N'N'-dimethylcarbamate Hydrochloride—A mixture of 15.2 Gm. (0.1 mole) of β-chloroethyl dimethylcarbamate (3) and 24.2 Gm. (0.2 mole) of benzylmethylamine was heated at 100° for 1 hr. After cooling, the solid reaction mixture was extracted with ether, the combined extracts concentrated, and the residue fractionally distilled to give 15.1 Gm. (64%) of ester, b.p. 110–120°/0.25 mm. The ester was dissolved in anhydrous ether and precipitated as the hydrochloride salt by the addition of an ethereal solution of hydrogen chloride. The resultant oil crystallized on standing and was recrystallized from an ether–alcohol mixture. The product melted at 146°.

Anal.—Calcd. for $C_{13}H_{21}ClN_2O_2$: C, 57.24; H, 7.76. Found: C, 56.93; H, 7.19.

Preparation of N-Benzyl-N-methylaminoethyl-N'-butylcarbamate Hydrochloride—The compound was prepared as noted in the carbanilate series (1). The product melted at 122–123° and was obtained in 58.5% yield.

Anal.—Calcd. for $C_{15}H_{25}ClN_2O_2$: C, 59.88; H, 8.38. Found: C, 60.11; H, 8.05.

Preparation of N-α-Methylbenzyl-N-methylaminoethanol—1-Bromoethylbenzene was reacted with *N*-methylaminoethanol as described previously (1) to give *N*-α-methylbenzyl-*N*-methylaminoethanol (67%), b.p. 131–133°/5 mm.

Preparation of N-o-Chlorobenzyl-N-methylaminoethanol—*o*-Chlorobenzyl chloride was reacted with *N*-methylaminoethanol as previously described (1) to give *N*-*o*-chlorobenzyl-*N*-methylaminoethanol (85%), b.p. 112–120°/1.5 mm.

Preparation of N-Benzyl-N-methylaminoethylamine—Benzylmethylamine was reacted with ethyleneimine according to the method of Coleman and Callen (4) to give *N*-benzyl-*N*-methylaminoethylamine (73%), b.p. 80–93°/2 mm.

Biological Assay

Each compound was evaluated for its hypocholesteremic activity in male, Swiss Webster mice. Ten mice, weighing between 28–34 Gm., were used for each test compound. The mice were maintained on a fat-supplemented, cholesterol-free diet.[1] Test compounds were administered orally in a tragacanth suspension at a dose of 25 mg./Kg. b.i.d. At the end of the 11-day treatment period, animals were sacrificed and pooled tissue cholesterols[2] were determined by the Nury-Smith method (5). Percent change from controls was obtained by comparison with values obtained in an untreated control group of animals carried simultaneously in the experiment. Decreases in plasma or liver total cholesterol greater than 20% from control values without an increase of 8% in either end point, or decreases of 12% or greater in both end points were considered desirable biosignificant results.[3]

DISCUSSION

The types of compounds reported here have not been previously described as exhibiting hypocholesteremic activity in experimental animals. In this study their effect on both plasma and liver cholesterol is reported. It is considered desirable to lower cholesterol levels in one or both of these tissues without increasing either before concluding that a material has produced a desirable effect.

It is difficult if not impossible to ascribe the variable activities of the different members of this series to any specific portion of the molecule, but certain observations might be significant. As seen in Table VII, the presence of an aralkyl substitution seems advantageous, but it can be varied considerably. The *N*-benzyl, *N*-phenethyl, and *N*-phenpropyl amines show activity in lowering plasma and liver cholesterol (groups *A*, *B*, and *C*). However, the branching of the aralkyl group (group *D*) and substitution in the aralkyl group (group *E*) appear detrimental.

Substitution in the carbanilate ring markedly affects activity in this series of compounds. *Meta* and *para* substitutions produce compounds with a more consistent desirable activity than *ortho* substitution. Methyl substitution appears more favorable than the chloro or methoxy. Analogous ureas (group *I*) did not exhibit patterns similar to the carbanilate series. Here only the *p*-methoxy compound (53) appeared interesting. Even the aromatic ring was not essential, as seen by the activity of the butylcarbamate (48).

Increasing the bulk of the molecule to compounds of the dicarbanilate type (groups *F* and *G*) did not yield useful agents.

The results of this biological study indicate that both terminal portions in the molecules of the compounds reported contribute to the type of activity produced by them. Although several of these compounds do block *in vitro* biosynthesis of cholesterol from mevalonate (7), their mechanism of hypocholesteremic action *in vivo* is unknown. If they should be acting by inhibiting cholesterol biosynthesis, it is tempting to suggest that the enzymatic site of activity has two or more foci of attachment. Such an implication has been suggested by Kraml *et al.* (8) when describing the hypocholesteremic activity of *N,N'*-dibenzylethylenediamine and related compounds.

[1] Vitamin free casein, 24%; sucrose, 45%; Alphacel, 4%; salt mixture (USP XIV), 4%; dextrin, 20%; vitamin fortification mixture, 2%; ethyl linoleate, 1%.

[2] It was found more convenient to use pooled rather than individual samples in this work because of the large number of assays necessary. The authors have found previously in groups of ten animals that the mean of ten individual assays did not differ measurably from the result obtained with a pooled sample.

[3] Triparanol tested under these conditions decreased serum cholesterol 17.8% and liver cholesterol 37.6%. Gas chromatography of tissue extracts of triparanol treated animals shows accumulation of desmosterol (6). No abnormal sterols were observed in the tissue extracts of the animals treated with the compounds of this report.

REFERENCES

(1) Barnes, R. W., and Rost, W. J., *J. Pharm. Sci.*, **51**, 146(1962).
(2) McKay, A. F., and Hatton, W. G., *Can. J. Chem.*, **31**, 772(1953).
(3) Carter, H. E., Frank, R. L., and Johnson, H. W., "Organic Synthesis," coll. vol. III, John Wiley & Sons, Inc., New York, N. Y., p. 167.
(4) Coleman, G. H., and Callen, R. E., *J. Am. Chem. Soc.*, **68**, 2006(1946).
(5) Nury, F. S., and Smith, E. R. B., *Clin. Chem.*, **3**, 110(1957).
(6) Holmes, W. L., and DiTullio, N. W., *Am. J. Clin. Nutr.*, **10**, 310(1962).
(7) DiTullio, N. W., unpublished data.
(8) Kraml, M., Humber, L. G., Dubuc, J., and Gaudry, R., *J. Med. Chem.*, **7**, 500(1964).