Hypocholesterolemic Indole-2-carboxylic Acids

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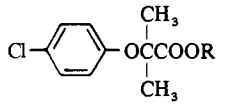
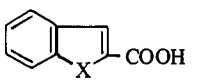
Received November 11, 1971

A series of indole-2-carboxylic acids was evaluated for effects on plasma lipids in rats. 5-Chloroindole-2-carboxylic acid (7) was found to lower plasma cholesterol; it was twice as potent as clofibrate and did not affect liver weight. Plasma cholesterol decrease was not accompanied by accumulation of sterol precursors of cholesterol biosynthesis. 7 did not affect plasma triglycerides nor did it reverse Triton-induced hyperlipidemia and thus differs from clofibrate. 7 did not inhibit norepinephrine-induced lipolysis in isolated rat fat cells and thus differs from nicotinic acid. 7 did not reduce plasma glucose and thus differs from 5-methoxyindole-2-carboxylic acid which was devoid of hypocholesterolemic activity. A high degree of specificity of biological action distinguishes these two agents from other indolecarboxylic acids.

A large number of alkylcarboxylic acids with aryl or aryloxy substituents have been reported to have hypocholesterolemic activity (for a recent review see ref 1). Clofibrate (**1**) is one of the most effective of these agents, and is the most widely used for control of hyperlipidemia.[2] This compound, in addition to reducing plasma or serum cholesterol, reduces circulating triglycerides and in rats produces liver enlargement.[3] We have evaluated the effects of coumarilic acid (**3**), benzo[*b*]thiophen-2-carboxylic acid (**4**), and indole-2-carboxylic acid (**5**) on blood lipids in rats. The heterocyclic acids retain certain structural features of the acid of clofibrate **2** in a cyclized form. Indole-2-carboxylic acid (**5**) was found to lower serum cholesterol in rats to the same extent and at the same dose as clofibrate.



1, R = C_2H_5
2, R = H



3, X = O
4, X = S
5, X = NH

A number of substituted indole-2-carboxylic acids and ethyl esters were then evaluated (Table I). Of all the possible chlorine-substituted congeners **7–12**, only 5-chloroindole-2-carboxylic acid (**7**) lowered serum cholesterol; 5-bromo- (**13**), 5-methyl- (**17**), and 5-ethylindole-2-carboxylic acid (**18**) also were active, but the 5-methoxy (**20**), 5-benzyloxy (**21**), and 5-nitro (**23**) congeners (in either free acid or ethyl ester form) were not. Since a number of indole derivatives are metabolized by oxidation at the 3 position,[4] several 3-substituted congeners (**9**, **16**, **22**, **24**) were evaluated, but no increase in activity was found. In addition to the compounds listed in Table I, indole-3-carboxylic acid,[5] indole-5-carboxylic acid,[6] 5-chloro-2-methylindole,[7] and 5-chloroindole[8] were evaluated and found to be inactive. Thus, structure-activity correlation clearly indicated that 5-chloroindole-2-carboxylic acid (**7**) is the best hypocholesterolemic agent of this series. Also evaluated and found to be inactive were: coumarilic acid (**3**),[9] benzothiophene-2-carboxylic acid,[10] 1-methylindene-2-carboxylic acid,[11] indene-1-carboxylic acid,[12] and quinoline-2-carboxylic acid.[13] No correlation could be found between apparent pK_a' values and hypocholesterolemic activity (Table I).

5-Chloroindole-2-carboxylic acid (**7**) lowered plasma cholesterol in immature and mature Sprague–Dawley rats and in Wistar rats (Tables II–IV). It was about twice as potent as clofibrate in immature rats. The decrease in plasma cholesterol was not accompanied by formation of desmosterol or other biosynthetic precursors of cholesterol. Unlike clofibrate,[14] **7** required repeated dosage and did not cause an increase in liver weight[3] (see Table II and Experimental Section). Plasma triglycerides were not affected (Tables III and IV). Triton-induced hyperlipidemia†,[15] was not reversed by **7** (Table III). Thus, the agent differs from clofibrate. Norepinephrine-induced lipolysis in isolated rat fat cells was not inhibited by 5-chloroindole-2-carboxylic acid (**7**) (Table V). Thus, the agent differs from nicotinic acid.[16] 5-Methoxyindole-2-carboxylic acid (**20**) was recently reported to reduce plasma glucose in fasted rats by inhibition of gluconeogenesis.[17] We found that **20** also produced a hypoglycemic effect in glucose-primed rats, while 5-chloroindole-2-carboxylic acid (**7**) had no effect (Table VI).

5-Chloroindole-2-carboxylic acid (**7**) inhibited weight gain in rats; the effect was dose related (Tables II and IV). Other active congeners (Table I) similarly inhibited body weight gain at the effective dose. Food intake was also decreased by **7**. Previous studies in this laboratory‡ have shown, however, that inhibition of weight gain through restriction of food intake will not produce significant reduction of plasma cholesterol in rats under these conditions. 5-Chloroindole-2-carboxylic acid (**7**) had no anorexic activity at 50 mg/kg po as determined by evaluation of eating behavior in mice.[18] The compound also was devoid of CNS effects in mice.§ **7** did not affect the weight of several organs (seminal vesicles, ventral prostrate, levator ani, testes, epididymal fat pad, adrenal, thymus, thyroid, pituitary, and spleen) in immature Sprague–Dawley rats after 10 daily administrations of 0.3, 3, 10 or 50 mg/kg sc, respectively. It was also devoid of uterotrophic and antiuterotrophic activity at 0.3, 3, 30 or 150 mg/kg sc, respectively, in mice.# As discussed above, liver weight also was not affected. Thus, no specific cause for the observed inhibition of weight gain could be found. Nonspecific toxicity as determined by LD_{50} in mice** was approximately 550 mg/kg po.§ Bauman and coworkers evaluated a number of analogs of 5-methoxyindole-2-carboxylic acid (**20**) for hypoglycemic activity in fasted rats, including **3**, **5**, **7**, **10**, **11**, **13**, **15**, **17**, and **23**.[19] Examination of their hypoglycemic data with our hypocholesterolemic data (Table I) indicates that **5**, **13**, **17**, and possibly **10** possess both activities, **3**, **11**, **15**, and **23** neither, while **20** is only hypoglycemic and **7** is only hypocholesterolemic. Thus it may be concluded that both nature and position of substitution determine specificity of biological action of indole-2-carboxylic acids.

†Also named tyloxapol, Triton WR-1339, or Triton A-20.

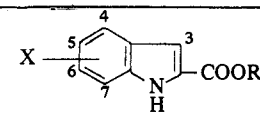
‡T. Kariya and T. R. Blohm, unpublished results.

§We thank Dr. A. Kandel and associates for these test result data.

#We thank Dr. A. C. Levy, T. H. Beaver, and associates for these test result data.

**Observation period 11 days; all deaths occurred within 24 hr.

Table I. Correlation of Structure and Hypocholesterolemic Activity of Indole-2-carboxylic Acids and Esters (Immature Charles River Sprague-Dawley Rats)



No.	X	R	Mp (lit. mp),[a] °C	pK_a'[b]	Plasma cholesterol reduction[c]
5	H	H	206–208 (200–201)[d]	5.04	+
6	H	C_2H_5	121–123 (125–126)[e]		±
7	5-Cl	H	288–289 (289–290)[f]	4.75	++
8	3-Cl	H	191–192 (180–182)[e]	5.00	–
9	3-Cl	C_2H_5	156–157 (153–154)[e]		–
10	4-Cl	H	258–259 (259–260)[g]	4.95	±
11	6-Cl	H	244–245 (242–244)[h]	4.95	–
12	7-Cl	H	233–236 (234–236)[f]	4.79	–
13	5-Br	H	276–277 (279–280)[i]	4.81	+
14	5-Br	C_2H_5	164–165 (165)[i]		+
15	1-CH_3	H	210–211 (212)[j]	5.21	–
16	3-CH_3	H	161–163 (165)[k]	5.74	+
17	5-CH_3	H	226–228 (229–230)[l]	5.11	+
18	5-C_2H_5	H	181–183 (184)[m]	5.09	+
19	5-C_2H_5	C_2H_5	100–103 (103)[m]		+
20	5-OCH_3	H	196–199 (196–197)[n]	5.00	–
21	5-$OCH_2C_6H_5$	C_2H_5	161–162 (162–164)[o]		–
22	3-NO_2	H	226–227 (230)[p]	3.61	–
23	5-NO_2	C_2H_5	217–218 (220–221)[q]		–
24	3-CH_2OH	Lactone	208–209 (208)[r]		–
25	3-$CH_2CH_2NH_2$	Lactam	185–187 (188–189)[s]		–
1	Clofibrate				+
2	Clofibrate, acid			4.81	+

[a]All materials analyzed correctly for C, H. [b]Apparent pK_a' in 1:1 EtOH–water by potentiometric titration (±0.1). [c]For methods see Experimental Section. (+) significant reduction (>20%) at 0.25% in diet; (++) significant reduction (>20%) at 0.125% in diet; (–) no significant reduction (<20%) at 0.25% in diet; (±) borderline activity 14–19% reduction at 0.25% in diet. [d]See ref 28. [e]See ref 29. [f]See ref 8. [g]See ref 30. [h]See ref 31. [i]See ref 32. [j]See ref 33. [k]See ref 34. [l]See ref 35. [m]See ref 36. [n]See ref 37. [o]See ref 38. [p]See ref 39. [q]See ref 40. [r]See ref 41. [s]See ref 42.

Table II. Effect of 5-Chloroindole-2-carboxylic Acid (7) and 2-(*p*-Chlorophenoxy)-2-methylpropionic Acid (2) on Plasma and Liver Cholesterol of Immature Sprague-Dawley Rats (Charles River) after 10 Days[a]

Compd	Number of rats	Daily dose		Plasma cholesterol		Liver		Body weight		
		% of diet	mg/kg	mg/100 ml	% reduction	g/100 g of body wt	Cholesterol, mg/g	Initial, g	Final, g	% inhibition of gain
7	6	0.0625	75	61.9 ± 4.46	12.3[c]	4.89	2.24 ± 0.048	75.2	134.5	13
	6	0.125	151	56.8 ± 4.35	19.5[d]	4.79	2.06 ± 0.075	75.3	130.2	20
	6	0.250	266	49.5 ± 1.61	29.9[d]	4.96	1.86 ± 0.039	75.2	110.7	48
	6	0.50	436	52.5 ± 5.82	25.6[d]	4.59	1.82 ± 0.044	75.3	87.5	82
2[b]	6	0.125	158	60.5 ± 4.31	14.3[c]	5.91	1.78 ± 0.089	75.3	138.3	8
	6	0.250	321	57.4 ± 2.73	18.7[d]	6.23	1.77 ± 0.028	75.3	145.7	0
	6	0.50	610	46.7 ± 4.40	33.8[d]	7.07	1.73 ± 0.028	75.2	126.3	25
Control	6			70.6 ± 3.39		4.83	2.04 ± 0.054	75.3	143.7	0

[a]See Experimental Section for detailed method. [b]Clofibrate acid. [c]Not significant ($p > 0.05$). [d]Statistically significant ($p < 0.05$).

Experimental Section††

Compounds. All compounds analyzed correctly for C, H. Identifying data are given in Table I. Compounds not listed there had the following melting points: indole-3-carboxylic acid, mp 210–212° (lit.[5] mp 214°); indole-5-carboxylic acid, mp 208–209° (lit.[6] 208–209°); 5-chloro-2-methylindole, mp 110–114° (lit.[7] mp 119°); 5-chloroindole, mp 52–60° (lit.[8] mp 71–72°); coumarilic acid, mp 194–195° (lit.[9] mp 192–193°); benzo[*b*]thiophene-2-carboxylic acid, mp 241–242° (lit.[10] mp 236°); 1-methylindene-2-carboxylic acid, mp 200–201° (lit.[11] mp 200°); indene-1-carboxylic acid, mp 157–159° (lit.[12] mp 156–157°); quinoline-2-carboxylic acid, mp 156–157° (lit.[13] mp 156°).

Biological Methods. Plasma Lipid Determinations. Rats were given free access to a diet containing the test compounds. This diet was prepared by evenly spraying commercial Purina Lab Chow (Ralston Purina Company, St. Louis, Mo.) with an ethereal or methanolic solution of the indicated amount of test compound and allowing the solvent to evaporate. Groups of 6 animals were thus treated for a period of 10 days along with an untreated control group. The daily food consumption of each group was measured by weighing to determine actual dose of test compound consumed. After 10 days, blood samples were obtained by cardiac puncture and the animals were sacrificed. The plasma was analyzed for cholesterol[20] and triglycerides[21] on a Technicon AutoAnalyzer. In a separate experiment, blood samples were obtained 3, 8, 24, 48, and 72 hr after administration of a single dose of 150 mg/kg po to immature Sprague-Dawley (Charles River) rats. No reduction of plasma cholesterol was observed. Clofibrate, under similar conditions, is reported to show an effect after 6 hr.[14] To determine whether sterol precursors of cholesterol biosynthesis were formed, plasma samples of animals treated with 5-chloroindole-2-carboxylic acid (7) at 0.25% in the diet were pooled and subjected to hydrolysis to convert sterol esters to free sterols, which were then extracted into hexane. Aliquots were converted to trimethylsilyl ether derivatives[22] and analyzed by glc using a 5% ECNSS-S (Applied Science Labs, Inc.) on 60–80 mesh Chromosorb W DMCS (Johns Manville) column, with cholestane as internal standard. Only one sharp peak, corresponding to cholesterol was observed. Desmosterol, $\Delta^{5,7}$-cholestadien-3β-ol, and other sterols that were previously shown to separate on glc under the conditions employed were found to be absent. A duplicate experiment confirmed this result.

Triton-Induced Hyperlipidemia (Table III). Following the procedure of Garattini, *et al.*,[15] groups of 10 animals were given free access to a diet containing the test compound in the indicated

††Melting points were determined on a Hoover capillary melting point apparatus and are corrected.

Table III. Effect of 5-Chloroindole-2-carboxylic Acid (7) and 2-(*p*-Chlorophenoxy)-2-methylpropionic Acid (2) on Plasma Cholesterol and Triglycerides of Mature Sprague–Dawley Rats (Charles River) after 11 Days and 20 Hr after Triton[a] (200 mg/kg iv in NaCl)[b]

Compd	Number of rats	Body weight, g		Food consumption, g/day av	Daily dose		Plasma cholesterol, mg/100 ml		Plasma triglycerides, mg/100 ml	
		Initial	Final		% of diet	mg/kg	Before Triton	After Triton	Before Triton	After Triton
7	10	297	303	12.8	0.125	53	51.6 ± 3.1 ($p < 0.003$)	183.3 ± 31.8 ($p < 0.25$)	79.7 ± 7.3 ($p < 0.2$)	1251.7 ± 231.4 (NS)
2[c]	10	313	346	15.1	0.25	114	36.5 ± 1.5 ($p < 0.001$)	124.3 ± 20.6 ($p < 0.007$)	60.3 ± 6.2 ($p < 0.03$)	480.0 ± 156.4 ($p < 0.03$)
Control	10	302	333	17.3			72.2 ± 5.2	234.0 ± 28.7	106.9 ± 17.5	1144.4 ± 215

[a] Also named tyloxapol, Triton WR-1339, or Triton A-20. [b] See Experimental Section for procedure. [c] Free acid corresponding to clofibrate.

Table IV. Effect of 5-Chloroindole-2-carboxylic Acid (7) and 2-(*p*-Chlorophenoxy)-2-methylpropionic Acid (2) on Plasma Cholesterol and Triglycerides of Immature Wistar Rats (Carworth Farms) after 10 Days[a]

Compound	Number of rats	Daily dose		Plasma cholesterol		Plasma triglycerides		Body weight		
		% of diet	mg/kg	mg/100 ml	% reduction	mg/100 ml	% reduction	Initial, g	Final, g	% inhibition gain
7	6	0.03	25	78.0 ± 3.52	0[c]	66.2 ± 10.54	0	77.7	110.8	25
	6	0.06	58	68.8 ± 2.31	9[c]	54.2 ± 6.24	0	78.8	110.2	29
	6	0.125	124	56.5 ± 2.25	25[d]	53.6 ± 8.59	0	78.0	105.7	37
	6	0.25	188	57.0 ± 1.85	25[d]	51.3 ± 8.14	0	78.7	88.5	78
2[b]	6	0.10	84	71.9 ± 3.39	5[c]	44.9 ± 7.27	8	78.8	118.8	9
	6	0.20	208	64.9 ± 3.60	14[c]	59.8 ± 6.18	0	78.7	116.5	14
	6	0.35	334	58.5 ± 2.24	24[d]	38.9 ± 3.02	20	78.8	105.8	28
	6	0.50	518	59.3 ± 2.63	22[d]	38.5 ± 4.02	21	78.8	112.2	24
Control	6			75.5 ± 3.24		48.8 ± 8.10		78.8	122.8	0

[a–d] See corresponding footnotes Table II.

Table V. Effect of 5-Chloroindole-2-carboxylic Acid and Nicotinic Acid on the Norepinephrine-Induced Release of FFA from Isolated Fat Cells[a]

Compound	Concn, *M*	FFA release,[c] μmoles/g of tissue	% inhibition
Control[b]		71.8 ± 5.0	
7	10^{-3}	69.5 ± 3.9	4
Nicotinic acid	10^{-3}	40.5 ± 1.8	43

[a] See Experimental Section for procedure. [b] Isolated fat cells were incubated for 4 hr in 2 ml of glucose-free medium containing norepinephrine, 0.1 μg. [c] Values are the mean (± SE) of 4 replicate flasks.

Table VI. Effect of 5-Chloro- and 5-Methoxyindole-2-carboxylic Acid (7 and 20) on Plasma Glucose of Glucose-Primed, Fasted, Intact Rats, 2 Hr after Oral Treatment[a]

Compound	Number of animals	Dose, mg/kg po	Plasma glucose, mg/100 ml[b]	% change from control
Control	10		94 ± 3	
7	5	100	112 ± 10	+12
20	5	25	104 ± 5	+11
20	10	100	74 ± 3	−21
Tolbutamide	10	25	77 ± 3	−18
Tolbutamide	10	100	57 ± 2	−40

[a] See Experimental Section for detailed procedure. [b] Values are means ± SE.

amounts. All animals, including the control group, also received a daily injection of olive oil (0.01 ml/kg sc) since another compound, not reported here, was also evaluated and administered subcutaneously in olive oil. After 11 days of treatment, 2-ml blood samples were obtained by cardiac puncture to give data before Triton. Triton† (200 mg/kg in 0.9% NaCl soln) was then injected intracardially. Twenty hours after Triton injection, blood samples were obtained for lipid determinations.

Plasma Glucose Determinations. The procedure described by Gerritsen and Dulin was used.[23] Young male rats of body weight 145–155 g (Charles River, C. D. strain) were fasted overnight (15 hr). The fasted animals were injected with 100 mg sc of glucose immediately after oral administration of the test compound in 0.5 ml of CMC vehicle.[24] Blood was withdrawn from the animals 2 hr after treatment, and plasma glucose was determined by the glucose oxidase procedure.[25] Tolbutamide was used as a reference compound.

Norepinephrine-Induced Lipolysis of Isolated Fat Cells. Isolated fat cells, prepared according to the procedure of Rodbell,[26] were obtained from rat epididymal adipose tissue and were used to study the effect of 5-chloroindole-2-carboxylic acid (7) on norepinephrine-induced lipolysis. FFA released into the incubation media was determined by the procedure of MacKenzie, *et al.*[27] Lipolysis was induced by the addition of norepinephrine at a concentration of 1 μg/ml to the incubation media. 7 was added at a concentration of 10^{-3} *M*.

Acknowledgments. We are indebted to Dr. Alfred Richardson, Jr., for suggesting this approach to us. The assistance of Messrs. Kenneth R. Hickey and Thomas A. Weil in synthesizing compounds **8**, **9**, **10**, **12**, **16**, **22**, **23**, and **24** by known procedures and in purification of compounds obtained from commercial sources, and the assistance of Mr. James R. Kincaid and Misses Nancy B. Beamer and Marcia K. Hackmann in the biological evaluations is acknowledged. We are indebted to Mr. M. J. Gordon and associates for microanalyses and determination of apparent pK_a's. We acknowledge with appreciation the interest and advice of Drs. R. W. Fleming and W. L. Kuhn.

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Synthesis and Biological Activity of Some 5-Substituted 2,4-Diamino-6-alkylpyrimidines. 3†

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Received November 15, 1971

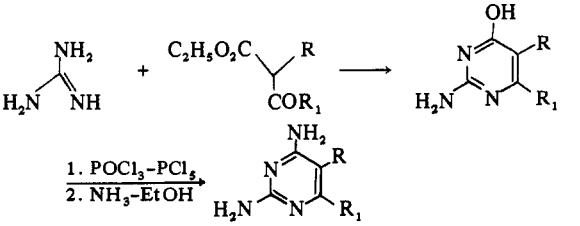
2,4-Diamino-6-methylpyrimidines having saturated straight-chain alkyl groups (C_3, C_5–C_8, C_{10}) in the 5 position were less potent growth inhibitors (ID_{50} 1.1 to 53 μM) of mouse mammary adenocarcinoma cells (TA3) *in vitro* than 2,4-diamino-5-(1-adamantyl)-6-methylpyrimidine (DAMP) (**15a**) (ID_{50} 6.0 nM). The ID_{50} values for another series of 2,4-diamino-6-methylpyrimidines having increasingly bulky 5 substituents decreased in the order 1-hexyl (**10a**) (2.5 μM), cyclohexyl (**14**) (0.40 μM), and 1-adamantyl (**15a**) (6.0 nM). The effects of two additional variables on the biological activity were investigated. 2,4-Diamino-5-(1-hexyl)-6-ethylpyrimidine (**16**) was 7 times more effective as an inhibitor than the corresponding 6-methyl analog (**10a**). The ethanesulfonic acid (ESA) salts of two diaminopyrimidines were 3 and 5 times more potent as growth inhibitors than the corresponding free bases. The best inhibitor of the entire study was 2,4-diamino-5-(1-adamantyl)-6-ethylpyrimidine ESA salt (**17**) with an ID_{50} of 0.25 nM which was about 30 times as active as methotrexate (ID_{50} 8.0 nM) when tested under the same conditions.

2,4-Diamino-5-(1-adamantyl)-6-methylpyrimidine[2] (DAMP) (**15a**) has been found to be a potent growth inhibitor of mouse mammary adenocarcinoma cells (TA3) *in vitro* and was subsequently shown to be a potent inhibitor of mammalian dihydrofolate reductase.[3]

It has been demonstrated by Baker, *et al.*,[4] that lipophilic substituents in position 5 of 2,4-diaminopyrimidines increase binding of these compounds to pigeon liver dihydrofolate reductase due to hydrophobic interactions. Since Nemethy and Scheraga[5] have shown that alkyl chains are coiled in aqueous systems, one would expect these groups to resemble the rigid adamantyl structure in such an environment. It was of interest, therefore, to prepare and test the biological activity of primidines with long alkyl chains at C-5.

Syntheses. The diaminopyrimidines were prepared by the standard route of condensation of guanidine with an appropriately substituted β-keto ester to form the 2-amino-4-hydroxy-5-substituted-6-alkylpyrimidines (Scheme I). The

Scheme I



physical constants for the 4-hydroxypyrimidines are given in Table I.

Baker, *et al.*,[4a] have previously reported preparation of **2** and **4** (Table I) by the same method used here. Another group[6] had described preparation of these pyrimidines by direct condensation of equal molar quantities of guanidine

†Presented in part before the joint ASPET-DMC meeting, University of Vermont, Burlington, Aug 24, 1971.[1] The synthetic work was supported in part by Grant CA-02906 and biological testing by CA-11047 from the National Cancer Institute of the U. S. Public Health Service.