MS 346.2147 (M, $C_{21}H_{30}O_4$). Anal. ($C_{21}H_{30}O_4$) C, H] and the α-methylene lactone **11** [28%; mp 139–140 °C; MS 304.2071 (M, $C_{19}H_{28}O_3$)], which were separated by preparative TLC eluting with chloroform–methanol (85:15).

3β-Hydroxy-16-methylene-17a-oxa-*D*-homo-5α-androstan-17-one (14). a. A solution of the lactone **13** (53 mg, 0.16 mmol), paraformaldehyde (30 mg, 0.8 mmol), and KOH (10 mg) in ethanol (1 mL) and water (0.5 mL) was heated under reflux for 10 min and diluted with water (3 mL). After it was left standing overnight at ambient temperature, the reaction mixture was cooled to 10 °C, acidified with dilute HCl, diluted with water (5 mL), and extracted with ether (three times). The combined ether extracts were washed with water, dried and evaporated. TLC of the residue, eluting two times with ether–chloroform (80:20), gave the lactone **13** (18 mg, 34%) and a less polar fraction which crystallized from methanol to give the α-methylene lactone **14** (15 mg, 27%): mp 259–260 °C; $[\alpha]_D$ +89°; IR (KBr) 3420 (OH), 1715 (C=O), 1630 (C=C) cm^{-1}; UV λ_{max} (EtOH) 214 nm (ε 3760); ^1H NMR δ 6.48 (1 H, m, i), 5.70 (1 H, m, ii), 5.63 (1 H, m, 3-H), 1.29 (3 H, s, 13-Me), 0.78 (3 H, s, 10-Me); MS 318.2241 (M, $C_{20}H_{30}O_3$).

b. A solution of the lactone **13** (136 mg, 0.44 mmol), 37% aqueous formaldehyde (0.5 mL, 4.84 mmol), diethylamine (150 mg, 2.05 mmol), diethylamine hydrochloride (170 mg, 1.55 mmol), and dioxane (10 mL) was heated under reflux for 8 h under a nitrogen atmosphere. The solution was made alkaline by the addition of sodium hydroxide (2 N) solution and extracted with benzene (three times). The combined benzene extracts were washed with NaCl solution, dried, and evaporated. TLC of the residue as in **a** gave the lactone **13** (45 mg, 33%) and the α-methylene lactone **14** (38 mg, 27%).

Reaction of L-Cysteine with 2-Methylene-4-oxa-5α-cholestan-3-one (10). A solution of the α-methylene lactone **10** (40 mg, 0.4 mmol) and L-cysteine (12 mg, 0.4 mmol) in 60% aqueous ethanol (5 mL) was heated under reflux for 1 h and allowed to cool to ambient temperature. The resultant white precipitate was collected by filtration and recrystallized from aqueous ethanol to give the cysteine adduct **15** (41 mg, 79%): mp 186–189 °C; IR (KBr) 3700–2300 (COOH and NH_2), 1725 (C=O of carboxylic acid and lactone), 1630 (CO_2^-) cm^{-1}; MS 402.3492 (M – $C_3H_5NO_2S$, $C_{27}H_{46}O_2$), 401.3388 (M – $C_3H_6NO_2S$, $C_{27}H_{45}O_2$), 400.3333 (M – $C_3H_7NO_2S$, $C_{27}H_{44}O_2$).

Cell Culture Assay. KB cells were cultivated in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum.

Preliminary screening was performed using a rapid microtitre-plate assay.[22] Active compounds were then assayed following the Cancer Chemotherapy National Service Centre (CCNSC) protocol for KB cells.[23] Cell numbers were determined using a Coulter counter, and LD_{50} values were determined from plots of mean cell counts at 48 h. In all cases, drugs were added in Me_2SO, which did not exceed a final concentration of 0.5%.

(22) E.-S. Huang, K.-H. Lee, C. Piantadosi, T. A. Geissman, and J. S. Pagano, *J. Pharm. Sci.*, **61**, 1960 (1972).
(23) *Cancer Chemother. Rep.*, **25**, 22 (1962).

Pyrido[2,1-*b*]quinazolinecarboxylic Acids as Orally Active Antiallergy Agents

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A series of 8-substituted pyrido[2,1-*b*]quinazoline-2-carboxylic acids was prepared by the nickel carbonyl mediated carboxylation of the corresponding bromides. The activities of these compounds in the rat PCA test are comparable to those of the corresponding 2-substituted pyrido[2,1-*b*]quinazoline-8-carboxylic acids.

Bronchial asthma is a chronic and debilitating disease which in its severe forms can be life threatening. Traditionally, three classes of drugs have been employed to combat the symptoms of this disease: β-sympathomimetic agents, phosphodiesterase inhibitors, and corticosteroids. All three classes have serious liabilities[1] and the introduction of disodium cromoglycate in 1967 was considered a major breakthrough.[2,3] This novel drug inhibits release, from sensitized mast cells, of the mediator substances responsible for the clinical manifestations of bronchial asthma while lacking bronchodilator activity.[3] Although disodium cromoglycate is not orally active and must be administered by insufflation, it has become an important drug in the prophylactic therapy of this state.

A great deal of synthetic work has been directed toward the discovery of more potent and orally active compounds. Recently, an interesting series of 2-substituted pyrido[2,1-*b*]quinazoline-8-carboxylic acids has been described.[4] We are therefore prompted to describe one aspect of our work in this area which constitutes an extension of the reported results.[4]

Results and Discussion

The 2-bromo derivatives **1–4** (Table I) were prepared by thermal condensation of 5-bromoanthranilic acid[5] with the appropriate 5-substituted 2-chloropyridine either neat or as a triglyme suspension in the presence of a catalytic amount of potassium iodide. The oxime **5** was readily available from the ketone **4** by reaction with methoxyamine. The 2-chloro-5-(1-methylethyl)pyridine (**17**) necessary for the preparation of **3** was synthesized through the intermediates **15** and **16** (Scheme I).

In order to convert the bromides **1–5** to the corresponding carboxylic acids, we employed a variation of a

(1) N. Svedmyr and B. G. Simonsson, *Pharmacal. Ther., Part B*, **3**, 397 (1978).
(2) H. Cairns, C. Fitzmaurice, D. Hunter, P. B. Johnson, J. King, T. B. Lee, G. H. Lord, R. Minshull, and J. S. G. Cox, *J. Med. Chem.*, **15**, 583 (1972).
(3) J. S. G. Cox in "Proceedings of Symposium of Disodium Cromoglycate in Allergic Airways Disease", J. Pepys and A. W. Frankland, Ed., Butterworths, London, 1970, pp 13–27.
(4) C. F. Schwender, B. Sunday, D. J. Herzig, E. K. Kusner, P. R. Schumann, and D. L. Gawlak, *J. Med. Chem.*, **22**, 748 (1979); C. F. Schwender, B. Sunday, and D. J. Herzig, *J. Med. Chem.*, **22**, 114 (1979).
(5) A. S. Wheeler and W. M. Oats, *J. Am. Chem. Soc.*, **32**, 770 (1910); A. S. Wheeler, *J. Am. Chem. Soc.*, **31**, 565 (1909).

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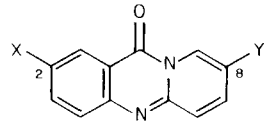
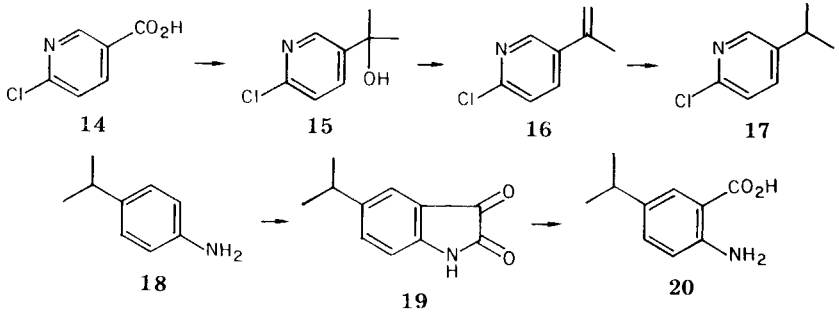
Table I. Pyrido[2,1-*b*]quinazolines



compd	X	Y	method	yield, %	mp, °C	solvent	formula	rat PCA: % inhibn,[a] 16 mg/kg ip	rat PCA: % inhibn,[a] 32 mg/kg po	rat PCA: ID_{50},[b] mg/kg po
1	Br	H	A	86	280-282	DMF-EtOH	$C_{12}H_7BrN_2O$			
2	Br	CH_3	A	48	277-281	EtOH	$C_{13}H_9BrN_2O \cdot HCl$			
3	Br	i-C_3H_7	A	25	191-194	CH_2Cl_2-Et_2O	$C_{15}H_{13}BrN_2O$			
4	Br	$COCH_3$[d]	A	23	221-224	CH_2Cl_2-Et_2O	$C_{14}H_9BrN_2O_2$			
5	Br	$CH_3C{=}NOCH_3$	B	88	210-213	CH_2Cl_2-Et_2O	$C_{15}H_{12}BrN_3O_2$			
6	CO_2H	H	C	48	354	DMF-HOAc	$C_{13}H_8N_2O_3$	16[e]		
7	CO_2H	CH_3	C	67	359	DMF-Et_2O	$C_{14}H_{10}N_2O_3$	69	81[f]	7.11[f] (5.21-9.47)
8	CO_2H	i-C_3H_7	C	78	314-316.5	CH_3OH	$C_{16}H_{14}N_2O_2$	81	92	0.026 (0.01-0.04)
9	CO_2H	$COCH_3$	C	37	305-309	CH_3OH	$C_{15}H_{10}N_2O_4$	44		
10	CO_2H	$CH_3C{=}NOCH_3$	C	68	324-327	THF	$C_{16}H_{15}N_3O_4$	36		
11	H	CO_2H	A	68	333[g]	DMF-EtOH	$C_{13}H_8N_2O_3 \cdot HCl$	17[e]		
12	CH_3	CO_2H	A	38	320	DMF-HOAc	$C_{14}H_{10}N_2O_3$	58	83	3.53 (1.48-5.60)
13	i-C_3H_7	CO_2H	A	27	327	pyridine	$C_{16}H_{14}N_2O_2$	76	87	0.45 (0.30-0.61)
disodium cromoglycate								46 (8 mg/kg)	18[f]	

[a] $p < 0.001$ using Student's t test. [b] Numbers in parentheses represent Fieller 95% confidence limits. [c] C: calcd, 47.96; found, 48.84. [d] The starting 5-acetyl-2-chloropyridine was prepared according to C. Räth and F. Schiffmann, *Justus Liebigs Ann. Chem.*, 487, 127 (1931). [e] Not statistically significant. [f] Ten-minute pretreatment time. [g] Lit. mp 323-325 °C (ref 4).



method originally developed by Cassar.[6] This procedure employs a catalytic amount of nickel carbonyl in DMF containing 1 equiv of calcium hydroxide under an atmosphere of carbon monoxide to effect carboxylation of aryl halides. When we applied these conditions to bromobenzene, no reaction occurred. However, when the reaction conditions were modified by the use of 5% aqueous DMF as solvent, an equimolar amount of nickel carbonyl, and 40 psi pressure of carbon monoxide, a 75% yield of benzoic acid was obtained. This modified procedure was applied to **1**–**5** to give the acids **6**–**10** (Table I).

The pyrido[2,1-*b*]quinazoline-8-carboxylic acids **11**–**13** were isolated from the reaction of the appropriate anthranilic acids with 6-chloronicotinic acid. The starting isopropylanthranilic acid **20** was prepared conventionally[7] through the isatin **19** starting with 4-isopropylaniline (**18**).

Compounds **6**–**13** were evaluated for antiallergy activity in the rat passive cutaneous anaphylaxis (PCA) test as described under the Experimental Section. The results, including those for disodium cromoglycate, are summarized in Table I. Four of the compounds (**7**, **8**, **12**, and **13**) have significant activity in the rat PCA test after oral administration. Comparable levels of activity are observed for both the 2-alkyl-8-carboxy and the corresponding 8-alkyl-2-carboxy analogues, suggesting that the nitrogen atom in the 10 position is not critically involved in binding to the active site and only plays a role in determining the conformation of the ring system. We note further that substitution of isopropyl for the methyl groups of **7** and **12** caused a marked enhancement of the oral activity of the products **8** and **13**, respectively. Compound **8**, moreover, ranks among the most active substances yet reported in the rat PCA test after oral administration.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Those over 310 °C were determined on a Dupont Instruments Model 900 thermal analyzer. Spectral data (IR, MS, and UV) were recorded for the new compounds and were in accord with the assigned structures. Microanalytical data were determined for C, H, and N on all new compounds and agree to within ±0.4% of the calculated values, except as indicated.

Method A. Synthesis of 2-Bromopyrido[2,1-*b*]quinazolin-11(11*H*)-one Hydrochloride (1). An intimate mixture of 100.0 g (0.880 mol) of 2-chloropyridine, 83.0 g (0.384 mol) of 5-bromoanthranilic acid,[5] and 1.0 g of potassium iodide was heated to a bath temperature of 145–150 °C overnight under a stream of argon. On cooling, the crude product was triturated with 150 mL of boiling ethanol and was collected to give 105.4 g (86%) of **1**, mp 280–282 °C dec.

Method B. 2-Bromo-8-[1-(methoxyimino)ethyl]pyrido[2,1-*b*]quinazolin-11(11*H*)-one (5). A solution of 1.2 g (3.78 mmol) of **4** and 1.2 g of methoxyamine hydrochloride in 85 mL of anhydrous pyridine was stirred at room temperature for 3 days. A yellow solid slowly precipitated. The pyridine was removed in vacuo and 100 mL of saturated sodium bicarbonate solution was added. After the solution was stirred briefly, the product was extracted with methylene chloride. The extract was dried and concentrated to yield a yellow solid (1.3 g). Crystallization from methylene chloride–ether gave 1.16 g (88%) of **5**, mp 210–213 °C.

Method C. General Procedure for the Carboxylation of Aryl Bromides. Caution! One should work in an efficient fume hood and observe appropriate safety precautions while working with nickel carbonyl. The reactions were carried out in the Fischer–Porter brand[8] of glass pressure bottles fitted with an efficient stirring bar and a head containing a pressure gauge and two ball valves, one leading via steel tubing to a carbon monoxide cylinder and the second capped by a rubber septum. In a typical experiment, a suspension of 0.04 mol of the aryl bromide and 0.041 mol of calcium hydroxide in 50 mL of DMF and 5 mL of water was placed in a 6-oz graduated Fischer-Porter pressure bottle, and the atmosphere was replaced with carbon monoxide by pressurizing the system to 30 lb and releasing the excess several times through a syringe needle piercing the rubber septum. Approximately 6 mL (0.046 mol) of nickel carbonyl was introduced via a 12-in. syringe needle welded directly to a control valve which is attached to the lecture bottle. This 12-in. needle passed through the septum and the orifice of one of the two ball valves. On completion of the addition, the needle was removed and the system was pressurized to 20 psi with carbon monoxide.

The resulting mixture was heated in an oil bath at 110–115 °C overnight and the internal pressure rose to 35–40 lb (at higher temperatures, the pressure continued to rise above 50 lb presumably due to decomposition of the reagent). On cooling, the reaction mixture was poured onto 250 mL of 1 N hydrochloric acid and stirred several hours to decompose the excess nickel carbonyl, and the resulting yellow solid was collected and recrystallized from the solvents indicated in Table I.

2-Chloro-5-(1-hydroxy-1-methylethyl)pyridine (15). Phosphorous oxychloride (75 mL, 0.8 mol) and phosphorus pentachloride (144 g, 0.69 mol) were added to 100 g (0.635 mol) of 6-chloronicotinic acid and intimately mixed. The reaction mixture was slowly heated in an oil bath to 80 °C over 25 min with stirring. The bath temperature was raised to 125 °C, and the solution was stirred and refluxed for 1 h. After the solution was concentrated under reduced pressure, anhydrous toluene was added and the solution was concentrated again, finally under high vacuum, to yield 6-chloronicotinoyl chloride as a colorless solid.

This acid chloride was dissolved in 600 mL of anhydrous ether and added dropwise over 2 h to a solution of methylmagnesium iodide prepared from 137 mL (2.2 mol) of methyl iodide and 50 g (2.06 g-atom) of magnesium in 700 mL of anhydrous ether. The reaction mixture was stirred and refluxed for 3 h. After pouring the cooled reaction mixture carefully into ice and 200 mL of acetic acid, the aqueous layer was made basic (pH 9) with 425 mL of 6 N sodium hydroxide. The ether was separated and the aqueous layer was saturated with sodium chloride and extracted four times with ether. After drying the combined extract over anhydrous magnesium sulfate, the extract was concentrated in vacuo to a yellow solid (112 g). Crystallization from ethyl acetate–hexane gave 44.5 g (26%), mp 70–74 °C, of **15** in the first crop. A second crop of **15** [46.7 g (27%), mp 67–71 °C] was obtained from ether–hexane. Anal. ($C_8H_{10}ClNO$) C, H, N, Cl.

2-Chloro-5-(1-methylethenyl)pyridine (16). A solution of 92.6 g (0.54 mol) of **15**, 4.6 g of *p*-toluenesulfonic acid monohydrate, and 0.9 g of hydroquinone in 1.5 L of anhydrous xylene was stirred and refluxed under a Dean-Stark water separator for 4.5 h. The xylene solution was washed with saturated sodium bicarbonate solution and dried over anhydrous magnesium sulfate. The xylene was removed by distillation [40–48 °C (14 mm)] through a Claisen head. Distillation of the residual oil through a Vigreux column gave 75.6 g (91%) of **16**, bp 110–114 °C (8 mm). Anal. (C_8H_8ClN) C, H, N, Cl.

2-Chloro-5-(1-methylethyl)pyridine (17). A solution of 86.9 g (0.566 mol) of **16** and 8.7 g of platinum oxide in 1 L of ethanol was shaken at atmospheric pressure in a hydrogen atmosphere for 1 h 45 min. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield an oil. Distillation through a Vigreux column gave 76.0 g (86%) of **17**, bp 105–109 °C (8 mm). Anal. ($C_8H_{10}ClN$) C, H, N, Cl.

2-Amino-5-(1-methylethyl)benzoic Acid (20). A 12-L flask was charged with 1.92 kg of hydrated Na_2SO_4, 133.2 g (0.80 mol) of chloral hydrate, and 1.8 L of water. The mixture was heated to 55 °C as a solution of 100 g (0.74 mol) of 4-(1-methylethyl)-aniline (**18**) in 64 mL (0.77 mol) of concentrated HCl, and 440 mL of water was added followed immediately by a solution of 164 g (2.36 mol) of hydroxylamine hydrochloride in 740 mL of water. The reaction mixture was rapidly heated to 100 °C, the heat was

(6) L. Cassar and M. Foa, *J. Organomet. Chem.*, **51**, 381 (1973).
(7) F. C. Whitmore and E. R. Hanson, "Organic Syntheses", Collect Vol. I, Wiley, New York, 1941, p 327; F. D. Popp, *Adv. Heterocycl. Chem.*, **18**, 1 (1975).
(8) Available from Fischer & Porter Co., Warminster, Pa. 18974.

removed, and the mixture was stirred for 10 min before it was cooled to 20 °C with the aid of an ice bath. Dilution with 1 L of water gave 160 g of crude brown 4-(1-methylethyl)isonitrosoacetanilide.

A solution of 1.36 L of sulfuric acid was maintained between 65 and 70 °C on a steam bath as 160 g of 4-(1-methylethyl)isonitrosoacetanilide was added. The temperature of the resulting mixture was raised to 75 °C for 30 min and cooled to 20 °C, and the solution was poured onto 16 L of ice and water and filtered.

The resulting solid was dissolved in 1.62 L of water containing 180 g (4.5 mol) of NaOH and filtered, and 50 mL of concentrated HCl was added. The resulting solution was treated with 500 mL of 30% hydrogen peroxide over 1 h, the pH was adjusted to 3–5 with HCl, and the precipitate was collected to give 50.6 g (36%) of **20**, mp 84–92 °C. A sample of **20** obtained from ligroin from an early run gave satisfactory analytical and spectral data and melted 86–89 °C. Later, a crystal modification, melting point 130–131 °C (dichloromethane–hexane), was obtained. Anal. ($C_{10}H_{13}NO_2$) C, H, N.

Passive Cutaneous Anaphylaxis (PCA) Assay. Male Sprague–Dawley rats obtained from Charles River Breeding Laboratories were used in the PCA assay. Two methods were used to prepare serum IgE antibody which is specific for egg albumin. In the first method, homocytotropic antibody was obtained by injecting 180- to 200-g rats with 1.0 mg of egg albumin (Difco Laboratories, Detroit, Mich.) intramuscularly and 6.4×10^{10} organisms of *Bordetella pertussis* vaccine (Eli Lilly and Co., Indianapolis, Ind.) intraperitoneally. Serum collected from each animal 18–20 days after sensitization was pooled, separated into 1-mL aliquots, and kept frozen until further use.

In the second method, rats weighing 150–200 g were immunized by the intraperitoneal injection of 0.5 mL of *Bordetella pertussis* vaccine (Cannaught Laboratories, Willowdale, Toronto, Canada, 20 optical units/mL) and 100 μg of egg albumin (Nutritional Biochemicals Corp., Cleveland, Ohio). Sixteen days later, *Nippostrongylus brasiliensis* (3000 larvae/0.1 mL) was administered subcutaneously, and on the 21st day, 10 μg of egg albumin in 0.5 mL of normal saline was administered intraperitoneally. On the 30th day, blood samples were collected by heart puncture; the serum was separated by centrifugation and refrigerated overnight at 5 °C. After a 24-h period, the samples were assayed for antibody activity by the passive cutaneous anaphylaxis method. Those serum samples which produced an average wheal diameter of 3 mm or greater following an intradermal injection of 0.05 mL of a 1:50 dilution of serum in normal saline were pooled, divided into aliquots, and kept frozen until further use. The ability of the antiserum to produce a PCA reaction was shown to persist for 72 h and to be inactivated by heating at 56 °C for 4 h.

The passive cutaneous anaphylaxis assay was carried out in rats weighing 190–220 g in a manner similar to that of Goose and Blair.[9] Rat IgE antiovalbumin in serum was prepared as described above. The IgE antiserum was titered with normal saline so that injections of 0.05 mL produced skin wheal diameters which averaged 7 to 10 mm. After a 24-h sensitization period, 1 mL of an aqueous solution containing a mixture of 8 mg of egg albumin (Nutritional Biochemical Corp., Cleveland, Ohio) and 5 mg of Evans blue dye (Nepera Chemical Co., Inc., Harriman, N.Y.) was administered intravenously to each rat. Forty minutes after challenge, each animal was sacrificed by cervical dislocation, and the dorsal skin was removed for examination. The long and short axes of the blued area were measured on the inner surface of the skin with a metric vernier caliper (VWR Scientific Division of Univar, Rochester, N.Y.), and the average diameter was determined for each reaction. Test compounds were administered 5 min before the intravenous injection of the dye and antigen, except as indicated in Table I. Logarithmically spaced doses were administered orally at the time of peak activity to determine the dose that caused a 50% inhibition (ID_{50}) in the expected wheal size. The procedure was usually conducted on five animals in the control and each of the treatment groups. Statistical analysis of the test results was conducted by the Student's *t* test of control vs. various treatments. The ID_{50} and 95% limits were determined by a modification of the Berkson minimum logic chi square method.[10]

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(9) J. Goose and A. M. J. N. Blair, *Immunology*, **16**, 749 (1969).
(10) J. Berkson, *J. Am. Stat. Assoc.*, **48**, 565 (1953).