4-(2-Azabicyclo[2.2.2]octan-2-yl)-2,2-diphenylbutyrylhydrazine (17). A suspension of **12** (0.5 g) in 5% HCl (10 ml) was refluxed 1 h. After cooling, the solution was made basic (NaOH) and extracted with Et_2O. The Et_2O solution was dried (anhydrous Na_2SO_4), the solvent evaporated, and the crude product recrystallized (Et_2O–SKB) to give **17**: 0.26 g (65%); mp 117–119°. Anal. ($C_{23}H_{29}N_3O$) C, H, N.

***N*-4-(2-Azabicyclo[2.2.2]octan-2-yl)-2,2-diphenylbutyryl-*N'*-acetylhydrazine Hydrochloride (18).** A solution of **5g** (3 g) and concentrated HCl (6 ml) was stirred overnight at room temperature. The solvents were evaporated at reduced pressure and the gummy residue was stirred in a mixture of Et_2O and Me_2CO until crystallization was completed. The solid was filtered, washed with Me_2CO, and dried to afford pure **18**: 1.9 g (53%); mp 248–249.5°; NMR ($CDCl_3$) δ 2.0 (3 H, singlet, CH_3). Anal. ($C_{25}H_{31}N_3O_2 \cdot HCl$) C, H, N, Cl.

Biology. The inhibition of gastrointestinal (gi) propulsion was studied in the charcoal meal cecal test as adapted from techniques previously described.[14-16] In these experiments, the extent of gi propulsion was measured with the aid of nonabsorbable and visually identifiable markers such as charcoal. Six male Charles River mice, 20–25 g, were fasted in screen-bottom cages with water supplied ad libitum for 24 h prior to the test. The animals were intragastrically (ig) pretreated with the test compounds employing a 0.5% methylcellulose suspension. The total volume of the suspension administered to these animals was kept constant at 10.0 ml/kg. The control group received the same volume of 0.5% methylcellulose solution. Thirty minutes after compound administration, the animals were given a single 0.2-ml dose of 10% suspension of charcoal powder in 1.0% methylcellulose solution. The animals were sacrificed 3.5 h later, and the cecum was examined on an all-or-none basis. In 95% of all control animals, charcoal was present in the cecum. Antidiarrheal drugs, such as diphenoxylate hydrochloride, interfere with the gi propulsive activity, so the presence or absence of charcoal in the cecum provides a measure for the pharmacological responses to these compounds. Consequently, the median effective dose (ED_{50}) can thus be calculated using the logistic method of Berkson.[17]

The analgesic activities were assayed by a modification of the mouse tail clip method of Bianchi and Franceschini.[18] The group of mice ($N = 6$) used in the charcoal meal cecal test was subjected to a pressure-standardized artery clip approximately 1 in. from the base of the tail. Those mice that did not respond by turning and biting at the clip within 15 s were scored positive for analgesic effects, and a provisional ED_{50} was graphically estimated. The separation of CNS and inhibition of gi propulsion effects can thus be expressed as a therapeutic index (ED_{50} analgesia/ED_{50} cecal test).

Acknowledgment. We wish to express our sincere gratitude to Dr. H. W. Sause for generously providing several samples, to M. Moats, E. Phillips, and J. Haller for technical assistance, and to Mr. M. Scaros and his staff for the supply of several intermediates.

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Cinnoline-3-propionic Acids, a New Series of Orally Active Antiallergic Substances

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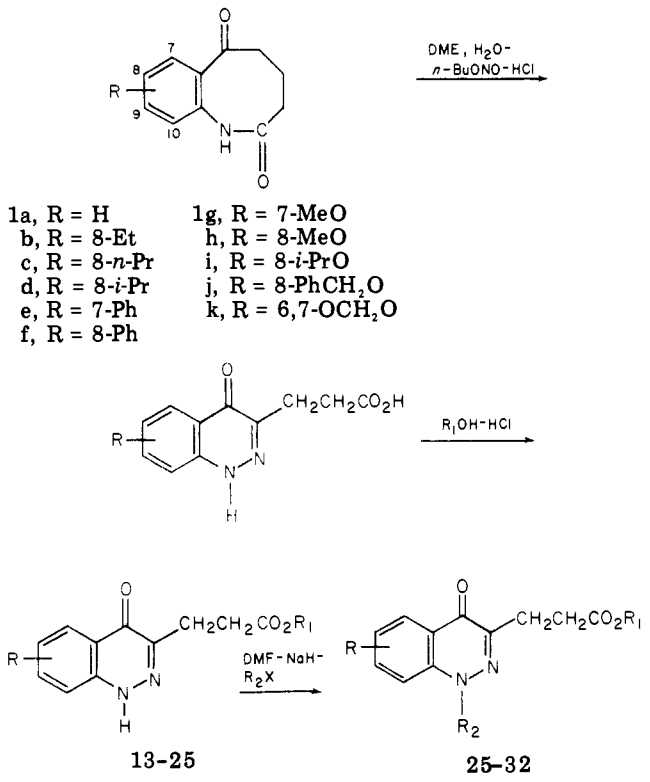
Received February 23, 1976

The synthesis of a series of 4-oxo-1,4-dihydrocinnolin-3-ylpropionic acids and their derivatives by a novel intramolecular Borsche-type cyclization from the corresponding substituted 4,5-dihydro-1*H*,3*H*-1-benzazocine-2,6-diones is described. These compounds show high activity in antiallergic bioassays and are of possible value in the treatment of asthma.

Disodium cromoglycate has been shown to be an effective and important drug for the treatment of certain types of asthma.[1] This drug is usually administered directly into the respiratory tract and, consequently, the search for an orally active replacement continues. Marked oral activity in humans has recently been reported for a number of series, namely, the 2-xanthonecarboxylic acids,[2] the 3-(5-tetrazolyl)thioxanthone 10,10-dioxides,[3] and the azapurin-6-ones,[4] and in animals for the nitroindan-1,3-diones[5] and the 3-(4-oxo-4*H*-1-benzopyran-3)acrylic acids.[6] During a chemical investigation of the oximation of 4,5-dihydro-1*H*,3*H*-1-benzazocine-2,6-dione (**1a**), an unexpected ring contraction was observed and the product was identified as 4-oxo-1,4-dihydrocinnolin-3-ylpropionic acid (**2**). On account of the structural resemblance of these rearrangement products to certain phenanthrolinecarboxylic acids[2] and their homologous relationship to 4-cinnolone-3-carboxylic acids,[8] the antiallergic potential of these compounds has been assessed from their relative activities in passive cutaneous anaphylaxis reaction (PCA)[9] in the rat using the heat-labile homocytotropic antibody. The methods of preparation and the biological activities in passive cutaneous anaphylaxis of the 4-oxo-1,4-dihydrocinnolin-3-ylpropionic acids, esters, and N-1 alkyl-

Scheme I



1a, R = H
b, R = 8-Et
c, R = 8-*n*-Pr
d, R = 8-*i*-Pr
e, R = 7-Ph
f, R = 8-Ph
1g, R = 7-MeO
h, R = 8-MeO
i, R = 8-*i*-PrO
j, R = 8-$PhCH_2O$
k, R = 6,7-OCH_2O

ated derivatives, by both the oral and intravenous route of administration, have been investigated.

Chemistry. This novel class of substituted 4-oxo-1,-4-dihydrocinnolin-3-ylpropionic acids and derivatives was prepared by the route outlined in Scheme I. The substituted 4,5-dihydro-1*H*,3*H*-1-benzazocine-2,6-diones (**1a–k**) were prepared by oxidative cleavage of the corresponding 1,2,3,4-tetrahydrocyclopent[*b*]indoles using sodium periodate in aqueous-tetrahydrofuran solution.[10] Reaction of the keto amides (**1a–k**) with butyl nitrite and gaseous hydrogen chloride in moist (~0.5% w/v) 1,2-dimethoxyethane gave the ring-contracted 4-oxo-1,4-dihydrocinnolin-3-ylpropionic acid (**2–12**). For a successful reaction the presence of traces of water in the solvent is essential; no rearrangement was observed in sieve-dried 1,2-dimethoxyethane. The structural identity of these products was confirmed by spectroscopic techniques and, in particular, by a comparison of the infrared, pK_a data, and ultraviolet spectra of the 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionic acid (**3**) with the parent 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylcarboxylic acid (**5**). The rearrangement of the substituted 4,5-dihydro-1*H*,3*H*-1-benzazocine-2,6-diones may be rationalized by invoking a hydrolytic cleavage of the amide bond and diazotization of the resulting aniline. The intermediate *o*-diazoacetophenone then partakes, probably through an enolic form, an intramolecular Borsche[11] cinnoline cyclization. On a number of occasions the diazonium intermediate could be isolated as a solid and was characterized by an intense infrared absorption at 2300 cm^{-1} ($N{\equiv}N^+$). The cyclization is assisted by the presence of electron-releasing substituents in position 6 of the cinnoline ring, whereas electron-releasing groups in a nonconjugative position inhibit the ring closure and the sole isolable product was the phenol obtained by hydrolysis of the diazonium intermediate. These structural electronic observations are consistent with a Borsch-type of cyclization mechanism.

The cinnoline acids (**2–12**) were readily converted to their respective esters (**13–25**) by refluxing in the appropriate alcohol acidified with gaseous hydrogen chloride. Alkylation of these cinnolone esters (**13**, **18**, **22**, and **24**) using sodium hydride and an alkyl halide in dimethylformamide invariably gave a single product. The methylation of 4(1*H*)-cinnolone gives 1-methylcinnolone and the anhydro base, methylated at position 2 in the ratio of 1:3.[12] The presence of a 3-substituent, however, leads to methylation largely at N-1 (whereas an 8-substituent favors reaction at N-2) suggesting that the process is governed mainly by steric rather than electronic factors. In contrast, methylation of 6,7-dimethoxy-4-oxo-1,4-dihydrocinnolin-3-ylacetic acid and esters[13] yields significant amounts (30%) of N-2 methylated products. The alkylated products obtained in this investigation were assigned structures as N-1 isomers for the following reasons: the infrared, ultraviolet, and NMR spectra of these compounds exhibit great similarity to those of the starting material; the NMR spectra show characteristic shifts for proton H-8 in the alkylated derivatives; finally, one would expect the less sterically hindered product to predominate. Catalytic hydrogenation of the cinnolinepropionic acid **2** in aqueous methanol using 5% palladium/carbon as catalyst gave a tetrahydro derivative in which the benzenoid ring was reduced.

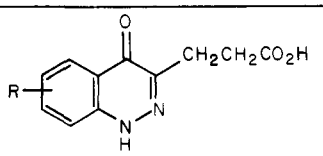
Biological Results. The compounds shown in Tables I and II were tested for their ability to inhibit the passive cutaneous anaphylaxis (PCA) reaction in rats. The more active and interesting members of the series, e.g., **3**, **14**, and **26**, have activities comparable with those of known clinically effective antiasthmatic agents such as disodium cromoglycate; additionally, oral activity of an interesting order was observed in this series notably for the N-1 methyl ester **26**. Among the 6-substituted acids (**2–5**, **7**, **9–12**) simple uncrowded alkyl substituents seemed preferable to electron-releasing methoxy and benzyloxy substituents. In contrast, the 6-isopropoxy group gives a compound (**10**) of moderate activity. Three dimensionally bulky groups were deleterious. On a limited number of examples substitution at positions other than six produced no improvement in activity. Most esters behave like their parent acids on intravenous dosing, presumably due to their rapid in vivo hydrolysis. Substitution at N-1 with a small alkyl group while greatly modifying the solubility properties of the molecule is nevertheless consistent with retained biological activity (e.g., **26**), in marked contrast to the analogous observations in the cinnolone-3-carboxylic acid series.

On account of the relationship of the cinnolone-3-propionic acids to the parent homologous carboxylic acid series of highly active PCA compounds, an effort was made to establish whether the activity of the propionic acid series was intrinsic or due at least in part to their metabolic conversion by β-oxidation to the cinnolonecarboxylic acid series. Ethyl 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionate (**14**) was administered to a rhesus monkey and the 24-h urine extract examined by TLC for the presence of 6-ethyl-4-oxo-1,4-dihydrocinnoline-3-carboxylate. No β-oxidized material could be detected; the dosed ester was mainly excreted as the corresponding acid (**3**).

Experimental Section

Chemical Methods. Substituted 4-Oxo-1,4-dihydrocinnolin-3-ylpropionic Acids (2–12). To a suspension of 1 g of 4,5-dihydro-1*H*,3*H*-1-benzazocine-2,6-dione (**1a**) in 20 ml of 1,2-dimethoxyethane and 0.1 ml of water was added 0.84 g of freshly prepared *n*-butyl nitrite. Hydrogen chloride gas was bubbled through the mixture at 25 °C for 5 min, and the resulting solution was stirred overnight at room temperature after which time the intermediate diazonium chloride had often separated. The solution or suspension was diluted with 10 ml of water, the

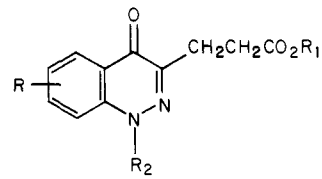
Table I



No.	R	Yield, %[a]	Crystn solvent	Mp, °C	Formula	Analyses	Act. in rat PCA test,[b] ID_{50}, μM/kg iv
2	H	61	Aqueous-EtOH	226-228	$C_{11}H_{10}N_2O_3$	C, H, N	37
3	6-Et	52	Aqueous-EtOH	240-241	$C_{13}H_{14}N_2O_3$	C, H, N	16[c]
4	6-*n*-Pr	54	Aqueous-HAc	230-231	$C_{14}H_{16}N_2O_3$	C, H, N	23
5	6-*i*-Pr	45	*d*	228-230	$C_{14}H_{16}N_2O_3$	C, H, N	77
6	5-Ph	40	*d*	262-263	$C_{17}H_{14}N_2O_3$	C, H, N	>68
7	6-Ph	71	Aqueous-HAc	306-310	$C_{17}H_{14}N_2O_3$	C, H, N	31
8	5-MeO	36	*d*	257-259	$C_{12}H_{12}N_2O_4$	N	>81
9	6-MeO	44	Aqueous-EtOH	256-258	$C_{12}H_{12}N_2O_4$	C, H, N	>81
10	6-*i*-PrO	89	Aqueous-HAc	210-213	$C_{14}H_{16}N_2O_4$	C, H, N	29
11	6-$PhCH_2O$	59	EtOH	245-247	$C_{18}H_{10}N_2O_4$	C, H, N	47
12	6,7-OCH_2O	54	*d*	>310 dec	$C_{12}H_{10}N_2O_5$	C, H, N	>76

[a] Yields reported refer to recrystallized or reprecipitated material except for compound 10. [b] The ID_{50} for disodium cromoglycate under identical experimental conditions was 6 μM/kg iv. [c] Oral activity corresponds with the reported intravenous activity for the compounds noted. [d] Product purified by precipitation of the acid from an aqueous solution of the sodium salt.

Table II



No.	R	R_1	R_2	Yield, %[a]	Crystn solvent	Mp, °C	Formula	Analyses	Act. in rat PCA test,[b] ID_{50}, μM/kg iv
13	6-Et	Me	H	75 (crude)	MeOH	173-174	$C_{14}H_{16}N_2O_3$	C, H, N	35
14	6-Et	Et	H	69	Acetonitrile	179-180	$C_{15}H_{18}N_2O_3$	C, H, N	14[b]
15	6-Et	*i*-Pr	H	68	Acetonitrile	182-183	$C_{16}H_{20}N_2O_3$	C, H, N	38
16	6-Et	*n*-Hexane	H	60	Acetonitrile	123-124	$C_{19}H_{26}N_2O_3$	C, H, N	*d*
17	6-*n*-Pr	Et	H	90 (crude)	Acetonitrile	175-177	$C_{16}H_{20}N_2O_3$	H, N; C[c]	21[b]
18	6-*i*-Pr	Et	H	73 (crude)	Acetonitrile	155-160 dec	$C_{16}H_{20}N_2O_3$	C, H, N	28
19	5-Ph	Et	H	73 (crude)	EtOH	161-163	$C_{19}H_{18}N_2O_3$	C, H, N	>62
20	5-Ph	*i*-Pr	H	70	*i*-PrOH	141-142	$C_{20}H_{20}N_2O_3$	N	>59
21	6-Ph	Me	H	62	MeOH	255-257	$C_{18}H_{16}N_2O_3$	C, H, N	23[b]
22	6-Ph	Et	H	91	EtOH	201-202	$C_{19}H_{18}N_2O_3$	C, H, N	*d*
23	6-*i*-PrO	Me	H	76	Acetonitrile	205-207	$C_{15}H_{18}N_2O_4$	C, H, N	*d*
24	6-*i*-PrO	Et	H	64	Acetonitrile	184-186	$C_{16}H_{20}N_2O_4$	C, H, N	>66
25	5,6-OCH_2O	Et	H	65	Acetonitrile	231-233	$C_{14}H_{14}N_2O_5$	C, H, N	65
26	6-Et	Et	CH_3	61	Petr ether[e]	92-94	$C_{16}H_{20}N_2O_3$	C, H, N	26[b]
27	6-Et	Et	$CH_2CH{=}CH_2$	61	Petr ether[f]	80-82	$C_{18}H_{22}N_2O_3$	C, H, N	51
28	6-Et	Et	CH_2Ph	62	Petr ether[e]	110-112	$C_{22}H_{24}N_2O_3$	C, H	>55
29	5-Ph	Et	Et	65	Petr ether[e]	128-131	$C_{21}H_{22}N_2O_3$	C, H, N	>57
30	6-Ph	Me	Me	98 (crude)	Petr ether[e]-EtOAc	130-132	$C_{19}H_{18}N_2O_3$	C, H, N	62
31	6-*i*-PrO	Me	Me	97 (crude)	Petr ether[e]-toluene	124-126	$C_{16}H_{20}N_2O_4$	C, H, N	49
32	6,7-OCH_2O	Et	Et	96 (crude)	Petr ether[e]-toluene	125-127	$C_{16}H_{18}N_2O_5$	C, H, N	*d*

[a] Yields reported refer to recrystallized material except where noted by crude. [b] Oral activity corresponds with reported intravenous activity for the compounds noted. [c] C: calcd, 66.64; found, 66.1. [d] Compounds were too insoluble for biological testing. [e] Petroleum ether, bp 60-80°. [f] Petroleum ether, bp 80-100°.

mixture warmed for 5 min on a steam bath, and the resulting suspension filtered. The solid residue was crystallized from aqueous ethanol; there was thus obtained 4-oxo-1,4-dihydrocinnolin-3-ylpropionic acid (2). Compound 2 exhibited uv absorption at λ_{max} 239 (4.15), 250 (4.06), 282 sh (3.60), 292 sh (3.60), 292 sh (3.60), 3.41 (4.07), and 356 nm (4.06); infrared absorption at ν_{max} 1711 (st, carboxylic C=O), 1572 (medium, amidic C=O), and 1545 cm^{-1} (v st, C=N); pK_a values in 50% aqueous acetone of 6.05 and 10.58; and M^+ at 218.0695 (calcd for $C_{11}H_{10}N_2O_3$, M 218.0691). In a similar manner using the appropriate substituted benzazocine derivative, the compounds in Table I were prepared.

Esters of Subsituted 4-Oxo-1,4-dihydrocinnolin-3-ylpropionic Acids (13-25). A suspension of 1 g of 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionic acid (3) in 10 ml of methanol was heated under reflux. Hydrogen chloride gas was bubbled through the mixture under reflux for 30 min. The mixture was then heated under reflux overnight, cooled, and diluted with 50 ml of water. The resulting suspension was filtered, and the solid

residue, crystallized from methanol, gave methyl 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionate (13), mp 173–174 °C. In a similar manner, starting with the appropriate propionic acid, there was obtained the following esters (13–25) described in Table II.

N-1 Alkylated Esters of Substituted 4-Oxo-1,4-dihydrocinnolin-3-ylpropionic Acids (26–32). Sodium hydride in oil (60%, 0.33 g) was washed free from oil with 3 × 5 ml of petroleum ether (bp 40–60 °C) and then suspended in 10 ml of dimethylformamide. Ethyl 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionate (14) (1 g) was added and the mixture was stirred at room temperature for 15 min after which time 0.5 ml of methyl iodide was added and the mixture stirred at 60–70 °C for 2 h. The mixture was cooled and poured into 100 ml of water and the resulting suspension filtered. The solid residue was crystallized from petroleum ether (bp 60–80 °C) and gave ethyl 6-ethyl-1-methyl-4-oxo-1,4-dihydrocinnolin-3-ylpropionate (26), mp 92–94 °C. The following N-alkylated derivatives (27–32) were obtained in a similar manner from the appropriate starting materials except that when the reaction was poured into 100 ml of water, instead of being filtered, the mixture was extracted with 3 × 100 ml of diethyl ether; the combined extracts were washed with saturated sodium chloride solution, dried ($MgSO_4$), and evaporated to dyness. The residue was crystallized from the appropriate solvent and in this way compounds 27–32 reported in Table II were obtained.

Catalytic Reduction of 4-Oxo-1,4-dihydrocinnolin-3-ylpropionic Acid (2). A solution of 1 g of 4-oxo-1,4-dihydrocinnolin-3-ylpropionic acid (2) in 140 ml of methanol and 40 ml of water was hydrogenated at STP using 1 g of 5% palladium/carbon and 0.4 g of platinum oxide as catalyst for 5 h. The catalyst was filtered off and the solvent evaporated. Crystallization of the residue from water gave 4-oxo-1,4,5,6,7,8-hexahydrocinnolin-3-ylpropionic acid (33): mp 226–228° (0.81 g); ν_{max} 1715 cm^{-1} (acid C=O); δ (Me_2SO-d_6) 2.9–2.1 (8 H, complex, benzylic CH_2 and CH_2 adjacent to carboxylic group) and 1.9–1.7 (4 H, complex, other $-CH_2-$); pK_a values in 50% aqueous acetone of 6.00 and 10.84. Anal. ($C_{11}H_{14}N_2O_3$) C, H, N.

Pharmacological Methods. The antiallergic activity of the test compounds shown in Tables I and II was assessed by their ability to inhibit passive cutaneous anaphylaxis (PCA) in rats. All compounds were initially tested by the intravenous route at 20 mg/kg, while only the more active members were further evaluated by the oral route. Groups of three rats received an intradermal injection on one shaved flank of 0.1 ml of a suitable dilution of rat reaginic antiserum. After 48 h the animals were challenged with 1 ml of an equal volume of antigen (egg albumin, 10 mg/ml) and Evans blue 0.5% w/v. The intensity of the reaction was assessed 30 min later by awarding an arbitrary score from 0 to 10.[14] Compounds were administered either intravenously at the same time as antigen or orally 15 and 60 min prior to antigenic challenge. The normal screening doses are 20 mg/kg intravenously or 40 mg/kg per os. Results are expressed in terms of the ID_{50} as that dose required to produce a 50% inhibition of PCA compared with a control group of rats receiving no drug.

Metabolic Studies.[15] A capsule of the ethyl ester 14 was dosed to a rhesus monkey at 20 mg/kg and the 0–24 h urine collected, and the chloroform extract examined by TLC using the solvent systems chloroform–ethanol–formic acid (85:15:1 v/v) and 2-propanol–ammonia–water (7:3:1 v/v). No 6-ethyl-4-oxo-1,4-dihydrocinnolin-3-ylcarboxylic acid was detected colorimetrically or by removal from the plate by extraction with solvent and determination of the ultraviolet spectrum of the area where authentic 3-carboxylic acid was known to cochromatograph. The dosed ester 14 was mainly excreted in the urine as the corresponding acid 3.

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Structure of the Peptide Antibiotic Polypeptin

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Polypeptin, a basic peptide antibiotic isolated from *Bacillus circulans*, was separated into two components by countercurrent distribution. The two components, polypeptin A and polypeptin B, had identical amino acid compositions but varied in the structure of the hydroxy acid constituent attached to the α-amino group of the peptide chain. Polypeptin A contained 3-hydroxy-4-methylhexanoic acid and polypeptin B contained 3-hydroxy-5-methylhexanoic acid. The stereochemistry of these hydroxy acids was not determined. Studies involving partial acid hydrolysis and chemical synthesis are consistent with the lactone structure for polypeptin A. Polypeptin B differs only in the position of the methyl group in the hydroxyacyl moiety.

Polypeptin is a basic peptide antibiotic isolated[1] in 1948 from a variant of *Bacillus circulans*. It was originally given the name circulin after the producing organism but the name was later changed[2] because of a name conflict with another natural product from *B. circulans*. Polypeptin has a wide spectrum of antimicrobial action. It is active against many gram-positive and gram-negative bacteria and most fungi and originally attracted some interest because of its