O_7·HCl) C, H, N. The retention time of 1 was 8.0 min under the same conditions as the above HPLC analysis.

4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxoethyl]-3-[(trichloroacetyl)amino]butan-4-olide (11). To a solution of thoroughly dried 1 (10 g, 23.6 mmol) in pyridine (40 mL) cooled to 0 °C was added dropwise trichloroacetyl chloride (9.1 g, 50 mmol), and the reaction mixture was stirred for 4 h at 0 °C. After the pyridine had been evaporated in vacuo, the residue was dissolved in ethyl acetate (300 mL) and then extracted three times with 0.1 N aqueous HCl (200 mL). This was followed by washing with water (200 mL). The ethyl acetate layer was dried with Na_2SO_4 and evaporated to give 9.8 g of a white solid. The white solid was crystallized from ethanol/water to give 6.3 g (48%) of 11 in the form of white needles. A second crop was 2.1 g (16%). The samples were used without further purification, although TLC analysis showed a trace of another spot corresponding to the disubstituted deriviative. If the contaminant is the disubstituted derivative, both 11 and the contaminant will form the same compound if the deprotection procedure is followed. On TLC ($CHCl_3$/EtOH, 9:1) the R_f value of 11 was 0.57 and that of the contaminant was 0.62.

4-[1-Hydroxy-2-[[1-(3,4-dihydro-8-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-2-oxoethyl]-3-[(trichloroacetyl)amino]butan-4-olide (12). To a solution of 11 (6 g, 10.8 mmol) in 1,2-dichloroethane (60 mL) was added diazomethane (400 mmol) in ethyl ether, and the solution was stirred overnight. After the excess diazomethane had been destroyed with acetic acid, the solution was filtered, and the filtrate was dried in vacuo to give 5.75 g of crude 12, which was used without further purification: TLC ($CHCl_3$/EtOH, 9:1) R_f 0.60 and ($CHCl_3$/MeOH, 3:1) R_f 0.82; UV max (MeOH) 306 nm, 244.

6-[[1-(3,4-Dihydro-8-methoxy-1-oxo-1*H*-2-benzopyran-3-yl)-3-methylbutyl]amino]-4,5-dihydroxy-6-oxo-3-ammoniohexanoate (15). To the above crude 12 (5.5 g) in a solution of ethanol/water (1:1, 250 mL) was added dropwise 1 N aqueous NaOH with stirring until the pH reached 12. The pH was held constant at 12 by the addition of more of the same alkali. Alkaline hydrolysis for the purpose of deprotection was monitored with TLC until it had been completed. This step caused the opening of the γ- and δ-lactone rings. The UV absorption maximum of the solution shifted from 306 to 277 nm due to opening of the δ-lactone. To the above hydrolysate containing 13 was added methanol (20 mL) saturated with HCl gas (about 68 mmol) while cooling with ice. The mixture was stirred for 30 min and then dried in vacuo to give 14. Th residue was redissolved in ethanol/water (1:1, 100 mL). In order for the γ-lactone ring to be opened, 0.1 N aqueous NaOH was dripped into the above solution with stirring to a pH of 9.0. The pH was held at 9.0 by the addition of alkali until the spot of 14 could no longer be detected on TLC, and then the pH was adjusted to 6.5 with 0.1 N HCl. The resulting solution was passed through a column packed with Amberlite XAD-2 (300 mL) in water. The column was washed with methanol/water (1:4, 600 mL) and eluted with methanol/water (3:2, 500 mL). Fractions containing only 15 were combined and dried in vacuo to give 2.25 g of 15. The overall yield was 30.7%. The sample 15 obtained above showed UV max (MeOH) 306 nm (ε 3973), 244 (ε 5573); IR (KBr) 1720 (δ-lactone C=O), 1655, 1590, 1575 cm^{-1}; ^1H NMR (CD_3OD) δ 7.48 (dd, 1, J = 7 and 8 Hz, aromatic H), 6.84 and 6.98 (2 d, each 1, J = 8 and 7 Hz, aromatic H), 4.6–4.3 (m, 2, C_3 and $C_{5'}$ H), 4.19 (d, 1, J = 7 Hz, $C_{8'}$ H), 3.98 (m, 1, $C_{9'}$ H), 3.87 (s, 3, OCH_3), 3.66 (m, 1, $C_{10'}$ H), 3.1–2.8 (m, 2, C_4 H_a and H_b), 2.8–2.4 (m, 2, $C_{11'}$ H_a and H_b), 1.10–1.95 (m, 3, $C_{3'}$ and $C_{4'}$ H), 0.96 and 0.92 (2 d, 6, each J = 7 Hz, 2 CH_3). Anal. ($C_{21}H_{30}N_2O_9$) C, H, N. The R_f values of derivatives 13–15 on TLC were as follows: R_f ($CHCl_3$/MeOH, 3:1) for 13, 0.05; 14, 0.78; 15, 0.12; R_f ($CHCl_3$/MeOH, 1:1) for 13, 0.42; 14, 0.75.

Registry No. 1, 77674-99-8; 2, 86527-24-4; 3, 86527-25-5; 4, 86527-26-6; 4·Na, 86527-27-7; 5, 77675-03-7; 6, 86594-32-3; 7, 77675-00-4; 8, 77676-66-5; 9, 77676-74-5; 10, 86561-37-7; 11, 77675-97-9; 12, 77676-98-3; 13, 86527-28-8; 14, 86594-33-4; 15, 86527-29-9; 16, 77675-02-6.

Synthesis and Transport Applications of 3-Aminobicyclo[3.2.1]octane-3-carboxylic Acids

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The isomeric 3-aminobicyclo[3.2.1]octane-3-carboxylic acids were synthesized and compared with the widely used (1*R*,2*S*,4*S*)-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid as to specificity to the Na^+-independent membrane transport system L of the Ehrlich ascites tumor cell and of the rat hepatoma cell line HTC. The presence of an additional methylene group in the ring system leads to an optically symmetrical amino acid, with the advantages that the product is devoid of isomeric contamination. Hence, optical resolution is not necessary to secure a homogeneous test substrate for discrimination of amino acid transport systems. Through its inhibitory action on the cellular uptake of known system-specific amino acids, the bicyclo[3.2.1]octane amino acid proved more reactive than the bicycloheptane analogue with the Na^+-independent amino acid transport system of the test cells and not perceptibly reactive with the accompanying Na^+-dependent systems. Recent evidence of the presence of a second component of Na^+-independent amino acid transport, beyond system L, increases the importance of securing a variety of possibly discriminatory model substrates.

Metabolism-resistant amino acid analogues for a given membrane transport system have greatly assisted in the discrimination of the routes of uptake of each amino acid by various animal cells.[1-4] For example, N-methylation of 2-aminoisobutyric acid or alanine limits their uptake to Na^+-dependent system A. At the same time, this alteration eliminates their inhibition of uptake by other transport systems. A second Na^+-dependent system called ASC does not tolerate the *N*-methyl group but responds favorably to a side-chain hydroxyl or sulfhydryl group. System ASC is usually somewhat narrowed, relative to

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system A, in its scope among the neutral amino acids by limitation to a chain length equivalent to five carbons or less.[5] Because system A is so often at a repressed level, system ASC often dominates Na^+-dependent uptake of neutral amino acids. Furthermore, on protonation at lowered pH, it may even serve for transport of aspartate and its analogues of similar chain length.[6] Other Na^+-dependent systems limited to the transport of two or three amino acids occur in some but not all tissues.[4]

Na^+-independent amino acid transport appears to have a different physiological significance than that by the Na^+-dependent systems. As for the other components named, this component usually yields rectangular hyperbolas on kinetic analysis; i.e., it is saturable and inhibitable by selected analogues in a manner consistent with catalysis by a single mediating structure in the plasma membrane. This homogeneous mediation, broad in scope among the amino acids, is attributed to transport system L, which is generally less uphill or concentrative in its action (i.e., it tends to produce weaker amino acid gradients) than the Na^+-dependent systems.[1] It is relatively insensitive to hormonal regulation, participates to a large extent in physiologically important molecule for molecule exchanges of amino acids across the plasma membrane, and is under independent regulation. The more polar amino acids show less affinity for this system. This system appears to be the principal route for amino acids across the blood–brain barrier[7] and, hence, a likely critical point for nutritional blockade by elevated phenylalanine in phenylketonuria in infancy and in the distorted circulating amino acid patterns in hepatic encephalopathy. Side-chain bulk and apolarity in general only enhance the transport of amino acids by system L, which has led to its sometimes excessive association with the aromatic and branched-chain amino acids. The exceptional tolerance for molecular bulk has led to the strategy for experimental limitation of uptake of an amino acid to system L by crowding the side-chain region, as was first suggested by the improvement of specificity obtained on insertion of a 3-methyl group into 1-aminocyclohexanecarboxylic acid.[1] When the added carbon atom was anchored also to a second ring carbon, to yield the rigid system of 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (which as its amino endo isomer we designate BCH[8]), excellent specificity was obtained.

BCH continues to serve to discriminate the characteristic Na^+-independent component for neutral amino acids, despite the detection of additional Na^+-independent transport systems. Its stereoisomerism, at earlier stages a significant advantage for exploring the spatial relations at the biologically responsive sites,[4,6] now represents, however, a moderate technical disadvantage in that the biologically more reactive and system-specific levorotatory 1*R*,2*S*,4*S* isomer has not been made generally available. Although use of the racemic amino endo form has often been satisfactory, partial resolution no doubt occurs in various biological test situations. Introduction of another methylene group into the bridge bearing the amino and carboxyl groups to form the optically symmetrical homologue, the 3-amino endo isomer of 3-aminobicyclo[3.2.1]-octane-3-carboxylic acid (BCO),[10] has now avoided this problem. This change has approximately doubled transport reactivity without loss of transport specificity. The present results also show that it is no advantage to its specific transport reactivity that BCH has its amino and carboxyl group vicinal to a bridgehead carbon atom: i.e., that it presents a structural analogy to isoleucine as well as to leucine. Furthermore, as for the bicycloheptane analogue, the amino exo isomer (β-BCO)[10] is much less satisfactory than the endo form as a transport analogue. The advantage of the optical symmetry of BCO is of course not obtained with the isomeric 2-aminobicyclo[3.2.1]octane-2-carboxylic acid.[11] Previous studies on the achiral 2-aminoadamantane-2-carboxylic acid showed that it meets the usual requirements for inhibition of system L.[12] Study with the carboxyl ^{14}C-labeled form[13] yielded a usual K_i of 0.3 mM for BCH in inhibiting 2-aminoadamantane-2-carboxylic acid uptake by the Ehrlich ascites tumor cell and a tripling of its exodus rate by external BCH at 5 mM. MeAIB had insignificant effects on these fluxes, although exclusion from transport by system ASC was not established. Initiation of an unexplained reversal of its uptake after 3 min discouraged our use of this interesting structure as a transport model.[13]

Another development has increased the importance of comparing model substrates for the study of system L. The Na^+-independent transport of neutral amino acids has, on further study, uncovered a previously hidden heterogeneity in this transport component in human red blood cells[14] and a different heterogeneity that increases during primary culture of the rat hepatocyte.[15] These results mean that we must be prepared for the possible presence in other cells of two or more somewhat difficulty discriminated components of what has previously appeared to be a homogeneous system L transport. These distinct components might prove to have different physiological significance. The availability of BCO and the tropane amino acids,[16] along with further tests of such nominal system L substrates as 4-amino-1-methylpiperidine-4-carboxylic acid (MPA)[17] and azaleucine and thialysine,[18,19] may well facilitate observation of these suspected distinct transport-specific components in increased isolation. At the same time, the availability of new analogues may improve the opportunity to observe transport reactivity uncomplicated by the insulin secretogogue action seen so far with BCH only,[9,20] among these analogues. Artificial analogues of the type studied may also serve to assist the control of glucagon secretion, by artificially saturating system L in the blood–brain barrier to modify the access of tyrosine, tryptophan, valine, and other amino acids to the brain, for

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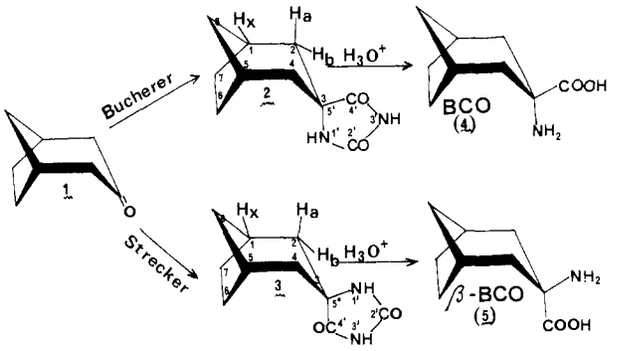
Scheme I



example, in hepatic encephalopathy, and to modify experimentally other amino acid flows from one organ to another.[21]

Chemistry and Structure. In a previous paper[22] we reported that the Bucherer–Bergs[23] reaction of tropinone furnished exclusively one spirohydantoin (the α-isomer), while theStrecker[24] reaction yielded exclusively another spirohydantoin (the β-isomer). The α-isomer had the 4′-carbonyl group of the spirohydantoin ring in the less sterically hindered equatorial (exo) position, while in the β-isomer this carbonyl group was placed in the more sterically hindered axial (endo) position. The configurational assignments were based on ^{13}C NMR data[22] and X-ray crystallography.[25] These results were in agreement with those reported by Edward and Jitransgri for anchored cyclohexanones.[26]

The assignments were in accordance with those expected from a detailed consideration of steric effects on either a thermodynamically determined product (the Strecker product) or a kinetically determined product (the Bucherer–Bergs product). These considerations showed that the spirohydantoin derived by the Strecker route (**3**) (Scheme I) should have its 4′-carbonyl group in the axial orientation and that the spirohydantoin derived by the conventional Bucherer–Bergs route (**2**) should have this group in the equatorial orientation (The EJ rule).[26]

In this case the stereoselectivity of the Bucherer–Bergs and Strecker reactions of bicyclo[3.2.1]octan-3-one (**1**) was also revealed, yielding two pure spirohydantoins, **2** and **3**, respectively. Configurations of **2** and **3** were established on the basis of spectroscopy data.

(1) A general, simple criterion to discover the isomeric purity of spirohydantoins in rigid systems is the study of the $N_{1'}$-H signal in the ^1H NMR spectra.[22] Thus, compounds **2** and **3** showed only one $N_{1'}$-H signal (δ 8.15 and 6.70, respectively) in their ^1H NMR spectra. Protons of the bicyclic system are affected by the different contribution of the diamagnetic anisotropic effect of the 4′-carbonyl group. Although the ^1H NMR spectra for these protons are rather complex, the equatorial (exo) position of the 4′-carbonyl group in compound **2** can be assigned by the study of the ABX systems formed by the bridgehead protons $H_{1(5)}$ and equatorial and axial protons $H_{2(4)}$. Axial H_a protons appeared in this compound at lower field (δ 2.28) than the equatorial H_b protons due to the deshielding effect of the 4′-carbonyl group, while in compound **3** the protons $H_{6(7)}$ are also affected, giving a more complex spectrum.

Table I. ^{13}C NMR (CF_3COOH) Chemical Shifts of Compounds 2 and 3

	chem shift, ppm downfield from Me_4Si	
	Bucherer product (2)	Strecker product (3)
$C_{4'}$	184.10[a]	184.24[b]
$C_{2'}$	161.61	161.00
$C_3(C_{5'})$	66.86	64.80
$C_2=C_4$	42.31	42.20
C_8	38.71	37.23
$C_1=C_5$	35.32	35.43
$C_6=C_7$	28.65	30.34

[a] Half-width of peak without spin decoupling = 15 Hz. [b] Half-width of peak without spin decoupling = 18 Hz.

(2) The isomeric purity and the stereochemical assignments of the spirohydantoins were also supported by the analysis of their ^{13}C NMR spectra (Table I). The 4′-carbonyl peak of **3** had a half-width of 18 Hz, as compared with a half-width of 15 Hz for this peak of **2**. These data reflect a greater vicinal coupling constant ($J_{^{13}C-^1H}$) in **3** than in **2**.

(3) Compounds **2** and **3** had different IR patterns. The frequency and absorbances of the N–H and C=O stretching absorption bands of **3** were similar to those of *cis*-bicyclo[3.3.0]octane-3-spiro-5′-hydantoin.[27] The X-ray crystallography[28] for the *endo*-4′-carbonyl isomer of this hydantoin showed a specific intermolecular association by hydrogen bonding. The different N–H stretching frequencies and C=O stretching absorbances in **2** must be explained by a different intermolecular association.

All the spectroscopic data so far analyzed confirm the isomeric purity and the stereochemical assignments of **2** and **3**.

Acid hydrolysis of **2** and **3** gave the amino acids BCO (**4**) and β-BCO (**5**), respectively (Scheme I). An IR study of these amino acids **4** and **5**, together with those derived from tropinone and pseudopelleterine, has been reported.[29]

Discussion

What is needed to establish the utility of BCO is to show that it is highly effective as an inhibitor of system L, as defined by the uptake of BCH, and ineffective in inhibiting the Na^+-dependent systems, as measured by the Na^+-dependent uptake of MeAIB (system A) and threonine (system ASC). Figure 1A shows that BCO (**4**), tested as an inhibitor of BCH uptake, is more reactive with the Na^+-independent transport system of the Ehrlich cell and of the hepatoma cell HTC (Figure 1B) than BCH. The half-saturating concentrations of the former are about half of those for the latter analogue. The β-isomer of BCO (**5**), tested to the limit of its solubility, was much less transport reactive in the Ehrlich cell than its epimer (Figure 1A).

The curve marked MPA in Figure 1B shows the inhibitory action of 4-amino-1-methylpiperidine-4-carboxylic acid[18] on the uptake of BCH by the hepatoma cell. This result may be compared with the activity of the two isomeric tropane amino acids,[16] which we may visualize as generated by the addition of a two-carbon bridge on one

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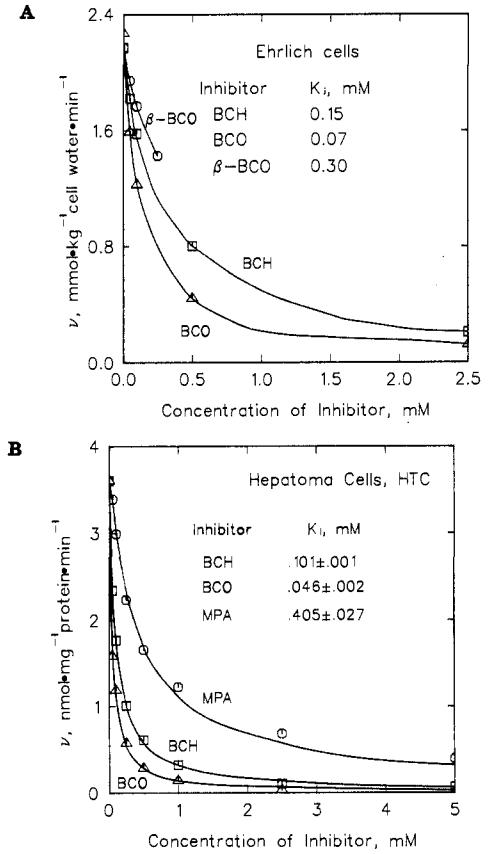

Figure 1. Inhibition of uptake of 0.1 mM L-[^{14}C]BCH into suspended Ehrlich cells and HTC cells in monolayers by structural analogues. Uptake of 0.1 mM BCH was measured for 1 min at 37 °C in the presence of increasing inhibitor concentrations. The concentration range for Ehrlich cells (Figure 1A) was 0 to 2.5 mM, and the concentration range for the HTC hepatoma cells (Figure 1B) was 0 to 20 mM, although data are not shown above 5 mM. The K_i of BCH on [^{14}C]BCH uptake is the same as the K_m of BCH for the given cell. BCO shows a K_i in fetal rat hepatocytes in primary culture similar to that in HTC.

side or the other of the methylpiperidine amino acid molecule. The K_i of the methylpiperidine amino acid as an inhibitor of the uptake of BCH is 1 mM,[18] whereas that observed for the more reactive of the tropane amino acids is 4 to 5 mM.[16] Our interest is intensified in the possible selectivity of these somewhat basic amino acid analogues, along with that of BCO,[10] between two or more so far difficulty discriminated components of uptake which may heretofore have been collected in various cells under system L.

Table II shows that BCO has no measurable inhibitory action on the uptake of MeAIB by the Ehrlich cell, showing its exclusion from system A.[5] A parallel test of the less effective β-epimer of BCO was restricted to a rather low concentration by its poorer solubility, a matter of little consequence, since its use as a transport substrate is not promising on the grounds of Figure 1A. System ASC shows a wider scope in rat hepatocytes or hepatoma cells than in the Ehrlich cell in the amino acids it accepts.[30] Hence, it is significant that threonine uptake by HTC also escapes inhibition (Table II).

Because of the above properties and because an optical resolution is not needed in its preparation to secure homogeneous BCO, we predict that it may replace BCH in most of their transport applications, especially if bicyclo-

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Table II. Effect of BCO (4) on the Uptake of 2-(Methylamino)[*carboxyl*-^{14}C]isobutyric Acid (MeAIB, 10^{-4} M) by Ehrlich Cells and of Threonine ($0.5 \cdot 10^{-4}$ M) by HTC Monolayers[a]

inhibitory analogue	concn, mM	MeAIB uptake, mmol/kg of cell water min^{-1}	Thr uptake, nmol/mg of protein min^{-1}
none	0	0.62	3.64
BCO	0.5	0.66	3.81
BCO	2.5	0.64	3.52
BCO	10	0.61	3.45
β-isomer of BCO	0.25	0.67	ND
MeAIB	20	0.035	3.04
Thr	10	ND	0.12

[a] The conditions are described in the Experimental Section. ND = not determined.

[3.2.1]octan-3-one becomes commercially available.

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR spectra have been recorded on a Bruker HX 90 and a Varian XL-200 (Me_4Si as internal reference). The ^{13}C NMR spectra were determined on a Varian FT 80. Infrared spectra were recorded on a Perkin-Elmer 577 spectrophotometer. Microanalyses were carried out with a Carlo-Erba 1108 instrument.

Bicyclo[3.2.1]octan-3-one (1) was prepared according to Jefford et al.,[31] using phase-transfer catalysis[32] for the dichlorocarbene addition step.

Bicyclo[3.2.1]octane-3-spiro-5′-hydantoins. (a) Bucherer Product (2). A solution of bicyclo[3.2.1]octan-3-one (1; 12.9 g, 0.10 mol), KCN (11.85 g, 0.18 mol), and $(NH_4)_2CO_3$ (29.95 g, 0.31 mol) in EtOH (52 mL) and water (52 mL) was placed in a sealed vessel and heated at 60–65 °C for 4 days. After the solution was cooled and acidified with 6 N HCl, 19 g (95% yield) of **2** was collected as white needles: mp 276 °C dec (from absolute ethanol); IR (KBr) 3335 (m), 3150 (w), 3030 (w) (NH), 1775 (m), 1725 (s, C=O) cm^{-1}; ^{1}H NMR (TFA) δ 9.62 (br s, 1 H, N_3, H), 8.15 (br s, 1 H, N_1, H), 2.52 (br s, 2 H, $C_{1(5)}$ H, $w_{1/2}$ = 12 Hz), 2.28 (2 d, 2 H, $C_{2(4)}$ H_a, $J_{a,b}$ = 4 Hz), 2.02–1.70 (m, 8 H, $C_{2(4)}$ H_b, $C_{6(7)}$ H_2, C_8H_2). Anal. ($C_{10}H_{14}N_2O_2$) C, H, N.

(b) Strecker Product (3). A mixture of 1 (10 g, 0.07 mol), KCN (6.02 g, 0.09 mol), and NH_4Cl (4.14 g, 0.08 mol) in ethanol (30 mL) and water (30 mL) was stirred at room temperature for 6 days. The solvents were removed in vacuo, the residue was extracted with ether, and the extract was dried over anhydrous Na_2SO_4. Passage of dry HCl gave 7.82 g (52% yield) of 3-aminobicyclo[3.2.1]octane-3-carboxynitrile hydrochloride. A solution of this crude aminonitrile hydrochloride (4.2 g, 0.023 mol) and KCNO (1.82 g, 0.02 mol) in acetic acid (17 mL) and water (2.5 mL) was heated under reflux at 100 °C for 1 h. The reaction mixture was heated under reflux with concentrated HCl (8.5 ml) for 15 min. The mixture was diluted with water (24 mL) and cooled, and the crude product was filtered off and recrystallized from ethanol to give 2.0 g (46% yield) of **3** as white crystals: mp >300 °C; IR (KBr) 3200–3100 (s, br, multiple bands, NH), 1775 (s), 1735 (s, C=O) cm^{-1}; ^{1}H NMR (TFA) δ 8.80 (br s, 1 H, N_3, H), 6.70 (br s, 1 H, N_1, H), 2.50 (br s, 2 H, $C_{1(5)}$ H, $w_{1/2}$ = 12 Hz), 2.28–1.62 (m, 10 H, $C_{2(4)}$ H_2, $C_{6(7)}$ H_2, C_8H_2). Anal. ($C_{10}H_{14}N_2O_2$) C, H. N.

3-*endo*-Aminobicyclo[3.2.1]octane-3-carboxylic Acid (4). Spirohydantoin **2** (10.0 g, 0.05 mol) was heated with 60% H_2SO_4 (54 ml) at 150 °C for 24 h. After cooling, the solution was neutralized with $BaCO_3$ (65.5 g) and filtered. The pure amino acid 4 was isolated by passage through 30.0 mL of ion-exchange resin (cationic Lewatit S-100) by using 0.25 N aqueous NH_3 as eluent and by removing the solvent in vacuo to give 6.7 g (84% yield)

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of 4 as white plates: mp >300 °C; IR (KBr) identical with authentic 4.[29] Anal. ($C_9H_{15}NO_2 \cdot {}^1/_2H_2O$) C, H, N.

3-*exo*-Aminobicyclo[3.2.1]octane-3-carboxylic Acid (5). Spirohydantoin **3** was treated as in **2** to give **5** (53% yield) as white plates: mp >300 °C; IR (KBr) identical with authentic 4.[29] Anal. ($C_9H_{15}NO_2 \cdot 1.5H_2O$) H, N; C: calcd, 55.07; found, 55.55.

Transport Methods. Ehrlich ascites tumor cells were propagated in Swiss white mice, separated, and washed in Na^+-free media.[33] We synthesized ^{14}C-labeled BCH[2] and MeAIB[34] from $Na^{14}CN$ and the corresponding ketones at specific activities in the range 3.3 to 50 Ci/mol. These are extensively studied preparations that yield no evidence for radiological impurity under tests with Ehrlich cell suspensions varying widely in density.[35] Their uptake was observed at 37 °C during 0.5 and 1 min, respectively, in 5% cell suspensions, the first in Na^+-free, choline-containing Krebs–Ringer bicarbonate medium and the second in the same medium containing Na^+, in a 5% CO_2–O_2 atmosphere, yielding a pH of 7.4. Uptake was terminated by dilution with ice-cold medium, followed by 2-min centrifugation at 200*g*. Adhering medium was blotted from the cell pellet before weighing.[1] Radioactive disintegrations in the separated suspending medium and in a sulfosalicylic acid extract of the cells were then counted for ^{14}C by liquid scintillation spectrometry.[1,36] Extracellular water was measured by the quantity of sucrose, provided in the medium, that was retained by the cell pellet. The uptake of the two amino acids in Figure 1A and Table II is recorded in millimoles per kilogram of cell water per minute.

Hepatoma cells of an HTC cell line,[37] propagated and extensively studied in our laboratory, were grown in a monolayer under a humidified atmosphere of 5% CO_2/95% air in Medium 199 (from GIBCO) at pH 7.4, containing 26 mM $NaHCO_3$, 62.5 μg/mL of penicillin, 5.8 μg/mL of streptomycin, 31.2 μg/mL of gentamycin, and 5 to 8% fetal bovine serum (Flow Laboratories). Three or four days before the transport test, cells were seeded in 24-well tissue culture cluster trays (Costar).[38] Transport was initiated by simultaneously adding to all test wells 0.25 mL of Krebs–Ringer phosphate medium (pH 7.4 and 37 °C) containing labeled BCH and a range of concentrations of carrier BCH, of BCO (4), of its β-epimer (5), or of 4-amino-1-methylpiperidine-4-carboxylic acid (MPA). After 1 min the medium was quickly decanted, and the cells were washed with 2 mL of ice-cold Na^+-free, choline-containing Krebs–Ringer phosphate medium.[37] The cells were then extracted with 220 μL of 5% trichloroacetic acid for 1 h. Radioactivity was then assayed by placing 200 μL of the extract in 2 mL of the scintillant 3a70B (Research Products International) and counting decompositions in a liquid scintillation spectrometer. The cell residues were dissolved in 200 μL of 1 N NaOH, and protein was assayed by a modified Lowry method[39] in the presence of 1% sodium dodecyl sulfate, with bovine serum albumin as a standard. The uptake rates are expressed in Figure 1B as nanomoles of test amino acid per milligram of protein per minute.

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Potential Antiatherosclerotic Agents. 2.[1] (Aralkylamino)- and (Alkylamino)benzoic Acid Analogues of Cetaben

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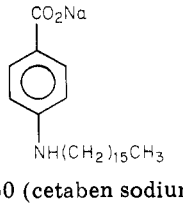
The syntheses of a series of (aralkylamino)- and (alkylamino)benzoic acids, as well as the corresponding esters and sodium salts, are described. The compounds were evaluated in vivo in rats for serum sterol and triglyceride lowering activity and in vitro for activity in inhibiting the principle cholesterol-esterifying enzyme of the arterial wall, fatty acyl-CoA:cholesterol acyltransferase (ACAT). Based on a combination of these two activities, cataben sodium (**150**) was selected for development as a hypolipidemic and potential antiatherosclerotic agent.

The syntheses of a group of alkoxybenzoic acids, as well as structure–activity relationships for their activity as hypolipidemic agents, have been reported;[2] however, the toxicity of these compounds has precluded their development as pharmaceuticals. As part of a continuing search for hypolipidemic and/or antiatherosclerotic agents of novel structure, a series of (alkylamino)- and (aralkylamino)benzoic acids, which were similar in lipophilicity to the alkoxybenzoic acids, was examined. As a class, these aminobenzoic acids were found to be less toxic than the related alkoxybenzoic acids, and one member of the series, cetaben sodium (**150**), was selected for further evaluation



150 (cetaben sodium)

as a hypolipidemic and potential antiatherosclerotic agent.[1,3-5] This paper begins a series of reports describing

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