2-Aminonorbornane-2-carboxylic Acid. Preparation, Properties, and Identification of the Four Isomers[1]

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Abstract: The four isomers of 2-aminonorbornane-2-carboxylic acid have been prepared for the study of spatial orientations at biological receptor sites. The configuration of one of these isomers has been determined by X-ray crystallography as reported in the accompanying paper. Synthesis by the Bucherer–Lieb procedure was found to yield mainly the isomer with the carboxyl group exo, whereas the Strecker procedure gave the isomer with the amino group in that orientation. The indication of the relative steric accessibility of the amino and carboxyl groups provided by ir spectra, titration, and hydrolysis rates of the *N*-formyl amino acids was, however, opposite to the expected one. A comparison of the reactivities of the four isomers in the inhibition of enzyme catalysis and of biological transport allowed the assignment of absolute configuration to those isomers not identified crystallographically. Confirmation of the assignment was obtained by synthesis of the model amino acid from optically enriched norbornanone and demonstrated that both levorotatory geometric isomers have configuration 1*R*,4*S*.

Our search for an amino acid analog specific to an apparently widespread Na^+-independent transport system L[2] was guided by the following considerations: (1) maximal tolerable bulkiness of side chain in all dimensions to prevent reaction with other transport systems; (2) maximally apolar side chain to produce high affinity, but without excessively low water solubility; and (3) maximal resistance to metabolic attack; *e.g.*, the α-carbon should be *tertiary*.

These considerations brought our attention to bicyclic structures and specifically to an amino acid constructed on the norbornane nucleus, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH).[3] This analog provided the additional advantages of structural rigidity and of existence as four isomers with small yet decisive differences in the distribution of their side-chain apolar mass. Synthesis by the Bucherer–Lieb and by the Strecker routes yielded different proportions of the racemic geometric isomers, which we arbitrarily designated a and b according to the sequence in which they are eluted from the resin columns of the amino acid analyzer by pH 4.25 citrate buffer.[4]

We have previously reported that a partially resolved mixture of BCH isomers proves an excellent discriminator for system L,[4] as well as a substrate for transport into *Escherichia coli*,[4] and a stimulator of insulin release from the rat pancreas.[5] The use of BCH should allow a description of structural requirements of substrates for certain biological receptor sites as well as a spatial description of the receptor sites themselves. The present paper reports the preparation, characterization, and identification of the four isomers of BCH as illustrated in Figure 1.[6]

Results

Although we previously reported an a-BCH/b-BCH ratio of 2.2 when the norbornane–aminonitrile mixture from the Strecker reaction run at 80° for 4 hr was hydrolyzed in boiling 6 *N* HCl,[4] Table I shows that the effect of temperature is critical to the isomer ratio and to the total yield of BCH obtained. Crystallization of the aminonitrile hydrochloride from hot acid solution always gave a product forming only a-BCH on hydrolysis. If, however, the crystalline aminonitrile plus 0.1 of the stoichiometric amounts of KCN and NH_4Cl were incubated in 50% aqueous methanol (final pH, 9.3) at 80° for 1 hr, the hydrolyzed product showed a ratio of 4.5.

Table I. Effect of Conditions of Reaction and Hydrolysis on the Geometric Isomer Ratio and Total Yield of BCH Synthesized by the Strecker Procedure

Reaction conditions,[a] Temp, °C (time, hr)	Ratio a-BCH/b-BCH	Total yield, %[b]
80 (8)[c]	4.3	96
80 (8)[d]	2.2	21
80 (2)[c]	3.3	49
55 (8)[c]	1.2	25
37 (8)[c]	0.23	6
4 (144)[c]	0.16	2

[a] One millimole each of norbornanone, KCN, and NH_4Cl was permitted to react in 10 ml of 50% aqueous methanol as described. [b] The amino acid solution formed on hydrolysis of the aminonitrile was brought to dryness and the sample applied to a well-calibrated column of the automated amino acid analyzer for the determination of total yield and isomer ratio. [c] The aminonitrile was hydrolyzed in 6 *N* HCl at 124° for 12 hr. [d] The aminonitrile was hydrolyzed in 6 *N* HCl at 109° for 4 hr.

The infrared spectra of the enantiomers obtained by the resolution of a-BCH and b-BCH all showed a broad absorption from approximately 2300 to 3600 cm^{-1}. Carboxylate stretching absorptions were seen at 1595 (doublet) and 1397 cm^{-1} in the case of a-BCH and at 1595 and 1376 cm^{-1} in the case of b-BCH. Amino acid I and II bands (N–H deformations[7]) at 1642 and 1529

(1) This work was supported by Grant HD01233 from the National Institute of Child Health and Human Development. H. S. T. received support through Training Grant GM00187 from the National Institutes of Health.
(2) H. N. Christensen, *Perspect. Biol. Med.*, 10, 471 (1967).
(3) The abbreviation BCH is used for 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid.
(4) H. N. Christensen, M. E. Handlogten, I. Lam, H. S. Tager, and R. Zand, *J. Biol. Chem.*, **244**, 1510 (1969).
(5) H. N. Christensen and A. M. Cullen, *ibid.*, **244**, 1521 (1969).
(6) R. S. Cahn, C. K. Ingold, and V. Prelog, *Angew. Chem. Int. Ed. Engl.*, 5, 358 (1966).
(7) L. J. Bellamy, "The Infra-Red Spectra of Complex Molecules," Wiley, New York, N. Y., 1954, pp 234–247.

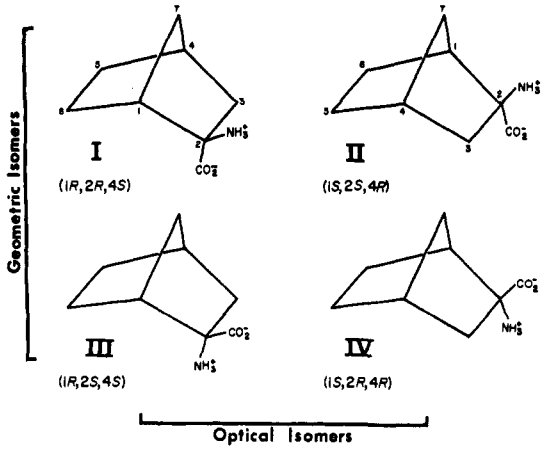

Figure 1. The four isomers of BCH. The designations for the optically active centers according to the Cahn-Ingold-Prelog convention[6] appear below each structure.

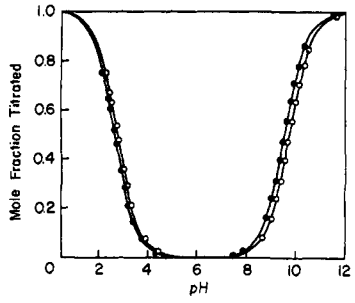

Figure 2. Titration curves for the geometric isomers of BCH. Closed circles are for (±)-a-BCH and open circles for (±)-b-BCH. The points are experimental and the curves, theoretical for the mean of the calculated values of pK_a'.

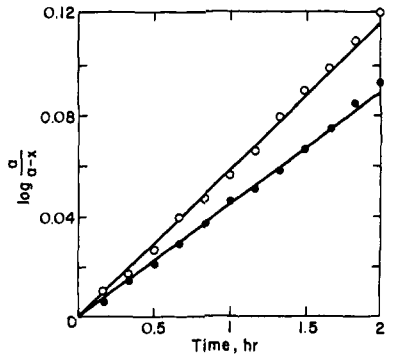

Figure 3. Pseudo-first-order rate plots for hydrolysis of the *N*-formyl derivatives of BCH. Closed circles are for (±)-a-BCH and open circles for (±)-b-BCH.

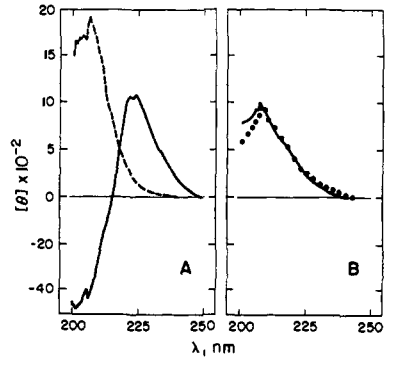

Figure 4. Spectra of the circular dichroism in water solution of A: (−)-a-BCH (solid line) and (−)-b-BCH (dashed line). B: BCH synthesized from norbornanone enriched in the levorotatory isomer, corrected to optical purity (solid line). This preparation contained 84% of the b isomer and 16% of the a isomer. The dotted line represents the theoretical spectrum for a solution containing the above percentages of the geometric isomers.

cm^{-1} were clear in the b isomer, but were almost undetectable in the broad absorptions of the a isomer.

The titration curves for both geometric isomers of BCH are shown in Figure 2. The calculated pK_a' values are 2.67 and 9.56 for (±)-a-BCH and 2.77 and 9.75 for (±)-b-BCH. The standard errors for these values are ±0.01 for the carboxylic acid pK_a' values and ±0.02 for the amino group pK_a' values. The pseudo-first-order rate constants for the hydrolysis of *N*-formyl-(±)-a-BCH and *N*-formyl-(±)-b-BCH in 1 *N* HCl at 50° are 0.1 and 0.13 hr^{-1}, respectively (Figure 3). The spectra of the circular dichroism for the levorotatory isomers of a- and b-BCH and for BCH synthesized by the Bucherer procedure from norbornanone enriched in the levorotatory isomer are presented in Figure 4 and will be discussed later.

Since BCH contains no α-hydrogen, it is not a substrate for L-amino acid oxidase; it is, however, an effective inhibitor of this enzyme. Figure 5 shows Lineweaver–Burk plots for the inhibition of L-leucine oxidation by L-amino acid oxidase using the four isomers of BCH. The inhibition constants are, (−)-a-BCH, 1.3 m*M*; (+)-a-BCH, 0.7 m*M*; (−)-b-BCH, 1.4 m*M*; and (+)-b-BCH, 1.4 m*M*. Notably, the inhibitions from (−)-a-BCH and (+)-b-BCH appear competitive, whereas those from (+)-a-BCH and (−)-b-BCH are apparently noncompetitive. Both types of inhibition however were eliminated by dialysis against Tris buffer pH 7.5.

We have also calculated the inhibition constants for the four isomers of BCH on the uptake of (±)-b-BCH-*carboxyl*-^{14}C into the Ehrlich ascites tumor cell and obtained the following values for the concentrations giving half-maximal inhibition: (−)-a-BCH, 2.4 m*M*; (+)-a-BCH, 0.3 m*M*; (−)-b-BCH, 0.05 m*M*; and (+)-b-BCH, 0.3 m*M*. These inhibitions were all apparently competitive.

Discussion

The crystallographic work of Apgar and Ludwig[8] shows that (−)-a-BCH is represented by structure I and therefore (+)-a-BCH by structure II. (±)-a-BCH is then the pair of isomers with carboxyl group endo and (±)-b-BCH, the pair with the same group exo. Isomerization during the Strecker reaction may well be a factor in the proportions of isomers a and b formed since heating the pure a aminonitrile under conditions very similar to those used in the overall synthesis resulted in partial conversion to the b form. The decrease in the ratio a-BCH/b-BCH from 4.3 at 80° to 0.16 at 0° (Table I) indicates a kinetic preference for the aminonitrile isomer with nitrilo group exo, although the isomer with that group endo appears to be the more thermodynamically stable one.

Our finding that the Bucherer synthesis of BCH yields a hydantoin which on hydrolysis gives a product con-

(8) P. Apgar and M. Ludwig, *J. Amer. Chem. Soc.*, **94**, 964 (1972).

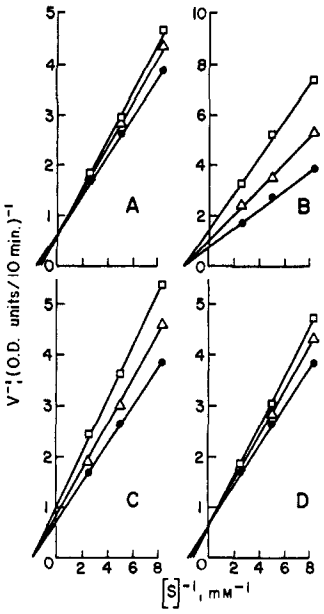

Figure 5. Lineweaver–Burk plots for the inhibition of L-leucine oxidation by L-amino acid oxidase using the four isomers of BCH. The inhibitors are, A, (−)-a-BCH; B, (+)-a-BCH; C, (−)-b-BCH; and D, (+)-b-BCH. ●, no inhibitor; △ and □, inhibitor present. See text for details.

taining 93% of the isomer with the carboxyl group exo[4] is consistent with the above results. The spirohydantoin is presumably locked into whichever configuration is taken first. The equilibrium ratio for the isomeric aminonitriles, however, must largely be a function of the position of the amino group since Wilder and Knight[9] have found little preference for the endo or the exo position in the equilibration of 2-nitrilonorbornane through a carbanion intermediate.

Substantial evidence suggests that the exo positions of the norbornane system are sterically more accessible than the endo positions.[10] The clarity of N–H deformation absorptions in the ir spectra of cyclic α-amino acids has been used as a criterion for the steric accessibility of the amino group, the more accessible group showing the better resolved spectrum.[11,12] We have found that the endo amino group in b-BCH shows the better resolved absorptions, although according to the usual view it should be more hindered.

The effect of steric environment on the dissociation constants of amino and carboxyl groups has been studied, and in general the former group is found to become more basic, and the latter group more acidic in a sterically favorable environment.[13,14] Our pK_a' values indicate in the case of BCH though, that it is the endo carboxyl group that is the more acidic, and the endo amino group that is the more basic. Our finding that the endo *N*-formyl group of the b isomer is removed at a rate approximately 30% greater than that for the exo group of the a isomer is consistent with our pK_a' values, although it is not consistent with the expectation of greater steric hindrance at the endo position.

(9) P. Wilder and D. B. Knight, *J. Org. Chem.*, 30, 3078 (1965).
(10) H. C. Brown, *Chem. Brit.*, 199 (1966).
(11) L. Munday, *J. Chem. Soc.*, 4372 (1961).
(12) R. J. W. Cremlyn and M. Chisholm, *ibid.*, 2269 (1967).
(13) R. D. Stolow, *J. Amer. Chem. Soc.*, **81**, 5806 (1959).
(14) C. W. Bird and R. C. Cookson, *J. Chem. Soc.*, 2343 (1960).

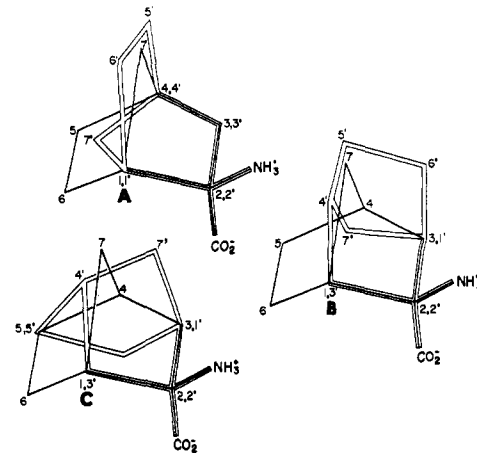

Figure 6. Attempted superposition of the isomers of BCH. Structures are drawn so that the amino and carboxyl groups occupy the same space. A, superposition of IV= on I—; B, superposition of III= on I—; and C, superposition of II= on I—. In each case the carbon numbers for I are unprimed, and those for the second isomer are primed. By reversing the positions of the amino and carboxyl groups in Figure 6A–C, the isomeric pairs III–II, III–I, and III–IV may be visualized respectively.

It remains somewhat of a puzzle that our data point to a greater steric accessibility for the endo position in BCH. On the basis of similar data on other cyclic amino acids, the major product of the Strecker synthesis has been assigned a structure with carboxylate group axial[11] or equatorial.[12] The use of these properties in assigning structures to amino acids appears therefore to be equivocal.

To assist in assigning absolute configurations to the (−)-b and (+)-b isomers, we examined the degree to which the four structures can be superposed on each other. Figure 6 illustrates the pairing of BCH isomers in spatial orientations that allow for superposition of the amino and carboxyl groups. The more notable differences between the two structures superposed in Figure 6A, namely isomers I and IV, are an apparent reversal of the positions of the one- and two-carbon bridges and a change in the dihedral angle N,C-2,C-1,-C-7 in I to N,C-2′,C-1′,C-6′ in IV. The structures are similar, however, in the general distribution of their side-chain apolar masses and in the positioning of the branch points, *i.e.*, the tertiary carbons. Superposition of II on I or III on I leads, however, to the side-chain masses of the amino acid pairs occupying largely different spaces (Figure 6B and 6C).

Our results for the inhibition of catalysis by L-amino acid oxidase indicate that the isomers (−)-a-BCH and (+)-b-BCH behave in much the same manner, as do the isomers (+)-a-BCH and (−)-b-BCH. In the Ehrlich cell, where all four isomers appear to be competitive inhibitors of transport by system L, (+)-a-BCH and (−)-b-BCH are better inhibitors than their respective antipodes, the difference in K_i's in each pair being at least sixfold. Transport behavior of BCH in the hamster gut is in agreement with its behavior in the Ehrlich cell; that is, the (+)-a isomer is more effective as an inhibitor than the (−)-a isomer, and the (−)-b isomer is more effective, either as a substrate or an inhibitor, than the (+)-b isomer.[15]

(15) Unpublished results.

On the basis of (1) the notable similarities of structures I and IV and structures II and III and (2) the biochemical similarities of the isomers (−)-a-BCH and (+)-b-BCH and the isomers (+)-a-BCH and (−)-b-BCH, we assigned structure IV to (+)-b-BCH and structure III to (−)-b-BCH; that is, the levorotatory geometric isomers differ only by their configuration at C-2. The partial reactivities of all four isomers of BCH in these systems may well be explained by the potential structural similarities of the isomers at least at the α, β, and β' carbons.

Since (−)-a-BCH and (−)-norbornanone have been shown to have the same absolute configuration,[8] the above assignment of isomers was confirmed by synthesis of b-BCH from an optically enriched sample of norbornanone obtained enzymatically. The spectrum of the circular dichroism of BCH synthesized by the Bucherer procedure from the ketone enriched in the levorotatory isomer is in close agreement with that expected if (−)-b-BCH and (−)-norbornanone also have the same configuration (Figure 4B). (−)-a-BCH and (−)-b-BCH have thus been shown to have configuration 1*R*,4*S*, thereby confirming the assignment of isomers based on the biological reactivities of the four isomers.

The present paper indicates the usefulness of the biochemical approach to the assignment of absolute configuration to compounds of organic chemical interest. Furthermore, the demonstration of the biochemical similarity of isomeric compounds of significantly different structure is of considerable importance in the study of spatial orientations at biological recognition sites, including those for catalysis, transport, and stimulation of hormone release. We are currently studying the biochemical reactivities of the four isomers of BCH in several other systems and will report these results elsewhere.[16]

Experimental Section[17]

BCH was prepared *via* the Bucherer route as described except that the spirohydantoin was hydrolyzed in a steam autoclave for 12 hr at 124°, and *via* the Strecker route as described except for two details: the reaction mixture was incubated for 8 hr at 80° and the aminonitrile was hydrolyzed for 12 hr at 124°.[4]

Preparation of (±)-2-Aminobicyclo[2.2.1]heptane-2-carboxynitrile Hydrochloride. (±)-Norcamphor (55 g, 0.5 mol) was dissolved in 250 ml of 50% aqueous methanol, and the solution was added to 32.6 g (0.5 mol) of KCN and 26.8 g (0.5 mol) of NH_4Cl dissolved in 250 ml of the same solvent. The sealed flask was incubated at 80° for 8 hr, after which the solution was acidified to pH 1 with 6 *N* HCl, evacuated, and brought to dryness on a rotary evaporator. The residue was dissolved in the minimal amount of boiling dilute HCl, the solution was decolorized, and crystals were allowed to form at 2°. Recrystallization yielded 35.3 g (34% of theoretical) melting at 215–217°. *Anal.* Calcd for $C_8H_{13}N_2Cl$: C, 55.64; H, 7.60; N, 16.23; Cl, 20.47. Found: C, 55.62; H, 7.61; N, 16.23; Cl, 20.59.

Preparation of (±)-a-BCH. The above aminonitrile (35 g) was dissolved in 500 ml of 6 *N* HCl, the solution was placed in a steam autoclave at 124° for 12 hr, the resulting solution was brought to dryness, and the residue was ground to a fine powder and exhaustively extracted with 1500 ml of methanol–ether, 1:1. The extract was brought to dryness, the residue was taken up in the minimum amount of water, and the solution was brought to pH 6.2 with 6 *N* NaOH. The slurry was allowed to stand at 2° overnight and then filtered, and the residue was recrystallized from water, yielding 59.2 g (77% of theoretical), melting at 322–324° with decomposition. Automated amino acid analysis revealed only one peak, corresponding to the more rapidly migrating a isomer.

Preparation of (±)-b-BCH. The pure b isomer was obtained by large scale ion-exchange chromatography of the geometric mixtures.[18] The preparation was homogeneous and melted with decomposition at 292–294°. (±)-b-BCH may be obtained directly in improved geometric purity by running the Bucherer–Lieb reaction in the cold over a period of several weeks.[4,19]

Preparation of *N*-Formyl Amino Acids. The *N*-formyl a and b isomers were prepared by the method of Sheehan and Yang.[20] The yields from the first crop of crystals were 70–75%. *N*-Formyl-(±)-a-BCH melted at 236–237° and *N*-formyl-(±)-b-BCH, at 209–211°.

Optical Resolution of (±)-a-BCH. *N*-Formyl-(±)-a-BCH (5.490 g, 0.03 mol) and 11.835 g (0.03 mol) of anhydrous *l*-brucine were dissolved in 50 ml of hot water. The solution was allowed to stand at room temperature for 4 hr and then at 2° for 4 hr. The crystals were collected and washed with 10 ml of ice-cold water, the mother liquor being set aside. The product was recrystallized from 50 ml of water, and this mother liquor was discarded. The yield of the less soluble *N*-formyl-a-BCH brucine salt was 8.40 g (48.6% of the total weighted components), melting at 136–137°; $[\alpha]^{25}D$ was −68.1°, 1% in methanol, and did not change upon recrystallization.

The salt (7.0 g) was dissolved in 50 ml of warm water and the solution was brought to pH 10 with 2 *N* NaOH with vigorous stirring. The resulting thick slurry was cooled to 2° and filtered. The residue was washed with three 10-ml portions of ice-cold water, and the brucine precipitate was discarded. The filtrate and washings were combined and brought to dryness, and the residue was refluxed in 6 *N* HCl for 2 hr, the hydrolysate then being brought to dryness. The product was dissolved in a minimum amount of water and further treated as described in **Preparation of (±)-a-BCH** to obtain the pure amino acid. The yield was 1.40 g (75% of theoretical for one optical isomer) melting at 309–311° with decomposition; $[\alpha]^{25}D$ was −61.4°, 1% in water and −47.9°, 0.5% in 5 *N* HCl. The CD in water (*c* 0.062%) had $[\theta]_{275}$ 0, $[\theta]_{225}$ + 1115, $[\theta]_{223}$ + 1056, $[\theta]_{216}$ 0, $[\theta]_{206}$ − 4837, and $[\theta]_{201}$ − 4818. The crystals of (−)-a-BCH·HBr used in the X-ray crystallographic studies reported separately[8] were obtained by allowing diethyl ether to diffuse slowly into an ethanol solution of the amino acid salt.

The mother liquor set aside from the first crystallization of the *N*-formyl amino acid brucine salt was treated as above to remove the brucine and obtain the pure amino acid. The yield was 1.45 g (77% of theoretical), melting at 309–311°; $[\alpha]^{25}D$ was +61.2°, 1% in water and +48.5°, 0.5% in 5 *N* HCl. The CD in water (*c* 0.062%) gave values for $[\theta]$ equal and opposite in sign to those reported for (−)-a-BCH.

Optical Resolution of (±)-b-BCH. For the resolution, we used our best preparation of BCH synthesized by the Bucherer method which contained approximately 97% of the b isomer. The amino acid was formylated as described.

The *N*-formyl amino acid preparation (20.90 g, 0.114 mol) and 45.05 g (0.114 mol) of anhydrous brucine were dissolved in 1000 ml of warm water, and the solution was allowed to stand at room temperature for 24 hr. The crystals were collected, washed, and recrystallized twice from 100-ml portions of water, the original mother liquor being set aside. The yield of the less soluble *N*-formyl-b-BCH brucine salt was 29.2 g (44% of the total weighed components), melting at 133–134°; $[\alpha]^{25}D$ was −2.7°, 1% in methanol, and did not change on recrystallization.

The amino acid was isolated as described under **Optical Resolution of (±)-a-BCH.** The yield was 6.7 g (78% of theoretical for one optical isomer). In both this and the following case determination of the melting point was prevented by rapid sublimation; $[\alpha]^{25}D$ was

(16) H. S. Tager and H. N. Christensen, *J. Biol. Chem.*, **246**, 7572 (1971); H. S. Tager and H. N. Christensen, *Biochem. Biophys. Res. Commun.*, **44**, 185 (1971); H. N. Christensen, B. Hellman, Å. Lernmark, J. Sehlin, H. S. Tager, and Täljedal, *Biochim. Biophys. Acta*, **241**, 341 (1971).

(17) Melting points were determined on a calibrated Fisher–Johns apparatus. The elemental analysis was performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. The following instruments were used: polarimeter, O. C. Rudolph Model 80; spectropolarimeter, Jasco ORD/UV/CD-5; infrared spectrophotometer, Perkin-Elmer Model 237; pH meter, Radiometer Type PHM4c; and amino acid analyzer, Phoenix Model K-8200-B. L-Amino acid oxidase (*Crotalus adamanteus*, purified), alcohol dehydrogenase (horse liver, purified), catalase (beef liver, purified), and NADH were purchased from the Sigma Chemical Co. (±)-Norbornanone was purchased from the Aldrich Chemical Co.

(18) H. S. Tager and R. Zand, *Anal. Biochem.*, 34, 138 (1970).

(19) Personal communication from Dr. Robert Zand.

(20) J. C. Sheehan and D. H. Yang, *J. Amer. Chem. Soc.*, **80**, 1154 (1958).

+24.4°, 1% in water and +21.2°, 0.5% in 5 *N* HCl. The CD in water (*c* 0.062%) had $[\theta]_{275}$ 0, $[\theta]_{206}$ − 1973, and $[\theta]_{203}$ − 1700.

The mother liquor from the first crystallization of the less soluble salt was concentrated to approximately 600 ml and allowed to stand at room temperature for 24 hr. The 2.3 g of crystals which appeared were discarded. The amino acid was isolated on further concentration of the solution, as already described. The yield was 7.1 g (84% of theoretical); $[\alpha]^{25}$ was −24.7°, 1% in water and −20.3°, 0.5% in 5 *N* HCl. The CD in water (*c* 0.062%) gave values for $[\theta]$ equal and opposite in sign to those reported for (+)-b-BCH. Both isomers were homogeneous on the amino acid analyzer, indicating that the contaminating a isomer had been removed.

Resolution of Norbornanone and Conversion to BCH. The resolution proceeded from the finding of Robertson and Hussein that norbornanone was slowly reduced by liver alcohol dehydrogenase.[21] (±)-Norbornanone (0.2 g), 1.4 g of NADH, and 0.4 g of horse liver alcohol dehydrogenase were dissolved in 200 ml of 0.03 *M* potassium phosphate buffer, pH 6.0, and the solution was incubated at 25° until the absorbance at 340 nm had decreased to 0.4 its original value. The reaction mixture was then rapidly heated to the reflux temperature and steam distilled. One volume of saturated aqueous potassium carbonate was added to the cooled distillate, and the milky solution was extracted several times with diethyl ether. The extracts were combined and filtered through anhydrous sodium sulfate, and the solvent was removed with a rotary evaporator. A solution in isooctane exhibited λ_{max} 295 nm, the previously reported value.[22] The CD in isooctane (*c* 0.0534%) showed $[\theta]_{316.5}$ + 334 and $[\theta]_{305}$ + 412 compared with $[\theta]_{316.5}$ + 1628 and $[\theta]_{305.4}$ + 1986 for the fully resolved ketone,[22] indicating an optical purity of 21% and identifying the compound as (−)-norbornanone.

The ketone (10 mg) was converted to BCH by the Bucherer procedure as described[4] except that the amino acid was not crystallized. A preparation containing 84% of the b isomer and 16% of the a isomer resulted. The CD spectrum corrected to optical purity (*c* 0.133% in water) is shown in Figure 4B.

pH Titration. The pK_a' values for the amino and carboxyl groups of both (±)-a-BCH and (±)-b-BCH were determined by titration of 20 ml of 0.025 *M* amino acid dissolved in 0.100 *N* NaCl with 0.1 *N* HCl and 0.1 *N* NaOH. These results were corrected for a blank titration and for dilution. The pK_a' was calculated for each point using standard equations.

Rate Constants for Hydrolysis of *N*-Formyl Amino Acids. Solutions (1.00 m*M*) of *N*-formyl-(±)-a-BCH and *N*-formyl-(±)-b-BCH were prepared in 1.00 *N* HCl at room temperature. Aliquots of 0.5 ml were added to individual test tubes, and the tubes were incubated at 50°. The reaction was stopped after selected time intervals by adding 0.5 ml of 1 *N* NaOH, and the tubes were placed on ice. The extent of hydrolysis was measured by ninhydrin assay of the liberated free amino acid.[23] All values were corrected for a zero-time blank. Pure a-BCH and b-BCH were used as standards, their extinction coefficients in the color reaction being indistinguishable.

Studies of L-Amino Acid Oxidase. Each 0.5-ml assay solution contained L-amino acid oxidase, 11.4 μunits (2.8 μg of protein); catalase, 1000 units; Tris (pH 7.5 with HCl), 20 μmol; and KCl, 50 μequiv. The oxidation of L-leucine at concentrations of 0.12, 0.2 and 0.4 m*M* was inhibited by the isomers of BCH at concentrations of 0.25 and 1.0 m*M*. After incubation at 30° for 10 min under air, the reaction was stopped by adding 0.5 ml of 0.05% 2,4-dinitrophenylhydrazine in 2 *N* HCl, and the tubes were allowed to stand for 10 min at room temperature. NaOH (1.0 ml, 2.5 *N*) was added and 10 min later the color read at 440 nm against a reagent blank. One absorbancy unit was equivalent to 0.13 μmol of α-keto acid, measured as pyruvate. Inhibition constants were determined by the method of Lineweaver and Burk.[24]

To test the reversibility of inhibition of L-amino acid oxidase by BCH, 0.28 mg of the enzyme was incubated at 30° for 10 min in 0.5 ml of 0.04 *M* Tris buffer pH 7.5, 0.1 *N* in KCl containing (1) no inhibitor, (2) 50 μmol of (−)-b-BCH, and (3) 50 μmol of (+)-b-BCH. Samples were separately dialyzed against two 400-ml changes of the same buffer, diluted 100-fold, and assayed as above using 5 m*M* L-leucine as substrate.

Studies on Transport into the Ehrlich Ascites Tumor Cell. The techniques used in these *in vitro* transport studies have been reported previously.[4] Uptake was measured from Krebs–Ringer bicarbonate buffer (143 m*N* in Na^+) under 95% O_2–5% CO_2 at 37°. Cells were allowed to take up the amino acid for 0.5 min. The uptake of (±)-b-BCH-*carboxyl*-^{14}C at concentrations of 0.056, 0.084, and 0.164 m*M* was inhibited by the isomers of BCH at concentrations as follows: (−)-a-BCH, 3.3 and 10 m*M*; (+)-a-BCH, 0.4 and 1.2 m*M*; (−)-b-BCH, 0.067 and 0.2 m*M*; and (+)-b-BCH, 0.4 and 1.2 m*M*. Rates of uptake were calculated and inhibition constants were derived using the method of Lineweaver and Burk.[23]

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