Convenient Synthesis of (*RS*)-4-Amino-3-hydroxybutyric Acid

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Abstract □ A new three-step synthesis of 4-amino-3-hydroxybutyric acid from an inexpensive starting material and under mild reaction conditions is described. Crotonic acid was brominated by the Wohl–Ziegler reaction to 4-bromocrotonic acid, which, in turn, was converted with ammonium hydroxide into 4-aminocrotonic acid. This compound, refluxed in water in the presence of a strong acid resin, afforded 4-amino-3-hydroxybutyric acid in good yields.

Keyphrases □ 4-Amino-3-hydroxybutyric acid—three-step synthesis from crotonic acid □ Anticonvulsants—4-amino-3-hydroxybutyric acid, three-step synthesis under mild reaction conditions

4-Amino-3-hydroxybutyric acid (III) was first synthesized by Tomita (1) in 1923 and was detected in the rat brain by Hayashi (2) in 1959. The remarkable importance of III stems from its biological function as a neuromodulator in the mammalian central nervous system (3, 4). Moreover, the metabolic correlation of III with 4-aminobutyric acid (5) and thus with glutamic acid and glutamine (6) justifies interest concerning its use in the treatment of epilepsy (7, 8).

In the elaboration of a research program on new 4-hydroxypyrrolidinones (9, 10), it was of interest to develop a versatile synthesis capable of giving fair amounts of III that could eventually be adapted to the preparation of some related compounds (*e.g.*, carnitine).

DISCUSSION

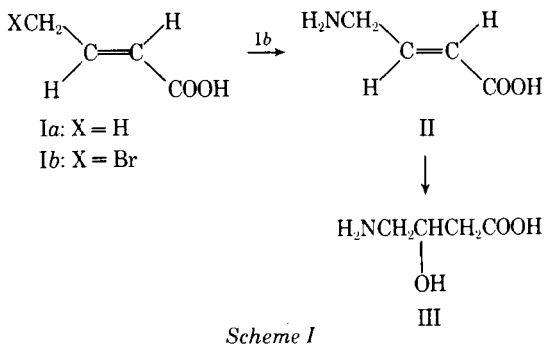
At present, essentially three methods exist for the synthesis of III: starting from material that contains two carbon atoms (*e.g.*, glycine), starting from compounds having three carbon atoms (*e.g.*, epichlorohydrin), and starting from compounds that possess the four-carbon framework (*e.g.*, ethyl acetoacetate).

The difficulties involved in the preparation of the key intermediate, 4-phthalimidocrotonic acid (11, 12), from phthalimidoacetic acid render the first method of limited synthetic utility. Another approach (1) consists of the reaction of phthalimide with epichlorohydrin to form 1-chloro-2-hydroxy-3-phthalimidopropane, exchange with alkaline cyanides, and final hydrolysis to III. In spite of its industrial application (13–15), this method suffers from serious ecological limitations due to the toxicity of epichlorohydrin and cyanides.

The four-step method involves bromination of the ethyl acetoacetate, reduction of the keto group, displacement of the halogen with ammonium hydroxide, and final hydrolysis of the ester (16). This method appears attractive because the material employed is inexpensive and the workup is safe. Unfortunately, the procedure is considerably limited by the low reactivity of ethyl 4-bromo-3-hydroxybutyrate to nucleophilic displacement, which causes a drastic fall in the overall yield.

To circumvent this problem, 4-bromocrotonic acid (I*b*) (17) was used. It possesses the 4-amino-3-hydroxybutyric acid framework and has an allylic bromine, which is highly reactive toward the nucleophiles.

Crotonic acid (I*a*, Scheme I) was brominated by the Wohl–Ziegler reaction with *N*-bromosuccinimide in boiling benzene in the presence of 2,2′-azobisisobutyronitrile as a radical initiator. An effort was made to find the best conditions for optimizing the transformation of I*a* into I*b*, thus avoiding the need of crystallization with severe loss of product (17). The replacement of the bromine by an amino group to yield 4-aminocrotonic acid (II) was performed with ammonium hydroxide in the presence of excess ammonium chloride to minimize the formation of secondary and tertiary amino derivatives (18).



Scheme I

The exchange, carried out in liquid ammonia, contrary to that reported (19), provided only very low yields of II, as could be expected from the poor reactivity of the anhydrous reagent at −33° (20). Addition of water to the double bond of II was catalyzed in boiling water by a strongly acidic resin[1]. This reaction can be regarded as the reverse of the known dehydration of III in warm sulfuric acid to form II (21).

This three-step synthesis of III (Scheme I) provided the final compound in 26% overall yield. Moreover, mild reaction conditions are employed in this preliminary method, which is still open to improvement.

EXPERIMENTAL[2]

4-Bromocrotonic Acid (I*b*)—To a solution of 20 g (0.23 mole) of I*a* in 200 ml of dry benzene, 45.6 g (0.25 mole) of *N*-bromosuccinimide was added under nitrogen. The solution was brought to a gentle reflux while stirring and was treated with 0.5 g (3.7 mmoles) of 2,2′-azobisisobutyronitrile. Refluxing was continued for 2 hr, and the solution was cooled to 10°. The resulting white precipitate was filtered off, and the filtrate was evaporated *in vacuo*.

The residue was taken up with 200 ml of carbon tetrachloride, and the mixture was cooled to 0° and filtered. The filtrate was evaporated *in vacuo* to give 38 g of a mixture of I*a* and I*b* as a brown solid. Examination of the ^{1}H-NMR signals of CH_3 at δ 1.95 ppm for I*a* and of CH_2Br at δ 4.05 ppm for I*b* indicated that about 85% (molar percentage) was the bromo derivative I*b*. An analytical sample of I*b* could be obtained upon several recrystallizations from light petroleum, mp 73–74° [lit. (17) mp 73.5–74.5°].

4-Aminocrotonic Acid (II)—To an ice-cold and stirred solution of 130 g (2.43 moles) of ammonium chloride in 1.3 liters of ammonium hydroxide (density = 0.89 g/ml), 38 g of the crude I*b* dissolved in 50 ml of methanol was added dropwise. The flask was sealed, and stirring was continued at room temperature for 16 hr. The resulting solution was concentrated *in vacuo* at 60° to remove excess ammonia and was then passed through a column (1.3 liters) of acidic resin[1].

The column was washed with water and developed with 5.8 liters of 5% ammonium hydroxide. The eluate was concentrated *in vacuo* to 80 ml and added dropwise to 1.5 liters of ethanol stirred at 0°. The resulting precipitate was collected and dried to yield 10.7 g (50%) of II, mp 157–160° dec. A further precipitation afforded an analytical sample, mp 174–176° dec. [lit. (11) mp 164° dec. and (21) mp 180° dec.].

(*RS*)-4-Amino-3-hydroxybutyric Acid (III)—To a solution of 10.7 g (0.106 mole) of II in 600 ml of water, 260 ml of acidic resin was added; the mixture was then refluxed with stirring for 16 hr. After cooling, the

[1] Amberlite IR 120

[2] Melting points were determined with a Büchi capillary melting-point apparatus and are uncorrected. ^{1}H-NMR spectra were determined on a Perkin-Elmer R-12B spectrometer in carbon tetrachloride, using tetramethylsilane as the internal reference.

resin was transferred to a column, washed with water, and developed with 1.3 liters of 5% ammonium hydroxide. The eluate was concentrated *in vacuo* to 25 ml, treated with active charcoal (5 g), and added dropwise to 1 liter of ethanol stirred at 0°. The precipitation was repeated, yielding 7.18 g (57%) of III, mp 204–206° dec. Recrystallization from water–ethanol afforded an analytical sample, mp 214–215° dec. [lit. (1) mp 214°].

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Vasodilator Activity of Adenosine Analogs

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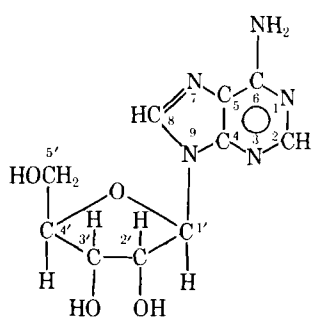
Abstract □ Various adenosine analogs were evaluated as smooth muscle vasodilators. The compounds were screened initially using a dog hindlimb preparation. Most analogs were less potent than adenosine. The three most potent compounds were tested for coronary vasodilator effects and duration of action on the isolated rabbit heart. 5′-Deoxy-5′-chloroadenosine and 5′-deoxy-5′-bromoadenosine were equipotent with adenosine but possessed a longer duration of action. 2′,3′,5′-Trideoxy-3′,5′-dichloroadenosine, an analog lacking both the 2′- and 3′-hydroxyl groups, had significant vasodilator activity.

Keyphrases □ Adenosine analogs, various—evaluated as smooth muscle vasodilators, dog hindlimb preparation □ Vasodilator activity—various adenosine analogs evaluated, dog hindlimb preparation □ Structure–activity relationships—various adenosine analogs evaluated as smooth muscle vasodilators, dog hindlimb preparation

The coronary vasodilator effects of adenosine (*Ia*) and its analogs have been recognized since 1929 (1). It has also been reported that these compounds cause the dilation of peripheral vessels (2, 3). It was suggested that adenosine is a physiological mediator of vasodilation (4). In all cases, the observed effects of adenosine are of short duration due to the rapid metabolism of the nucleoside to inosine (5) or phosphorylation to the corresponding nucleotide. Recently, Stein and coworkers (6, 7) reported that a series of esters and amides of adenosine-5′-carboxylic acid were potent cardiovascular agents that caused a marked increase in coronary sinus oxygen tension and prolonged



Ia

hypotension. These investigators postulated that these compounds act directly at an "adenosine receptor."

In an attempt to investigate the vasodilator activities of adenosine analogs further, a diverse series of adenosine analogs was synthesized and studied for peripheral activity in the hindlimb vasculature of the dog. Three of the most potent compounds were further studied on the Langendorff heart. The structural modifications included mono- and dihalo substituents in the sugar moiety, the extension of the 5′-position by one and two carbon atoms, an unsaturation in the carbon chain attached to the 5′-position, alterations in the sugar moiety, and a modification in the imidazole ring system. The vasodilatory effects of these compounds were compared to those of adenosine.