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Synthesis Of 3-Amino-3-Vinylpropanoic Acid And Its Conversion To 4-Amino-5-Hydroxy-4,5-Dihydrofuran-2-one Hydrochloride (HAD), A Cyclic Stabilised Form Of Aspartate 1-Semialdehyde Hydrochloride

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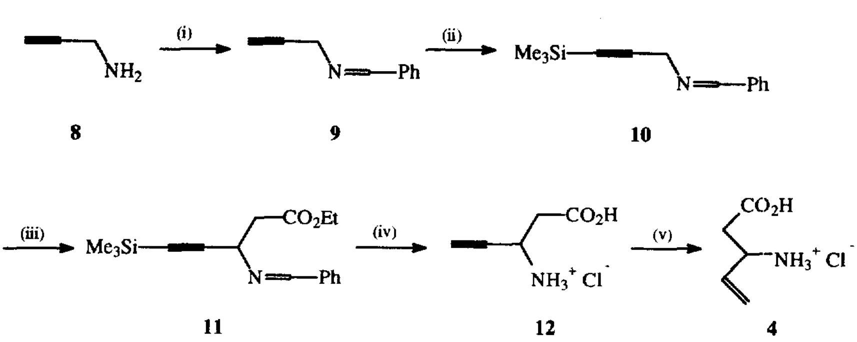
Abstract: 3-Amino-3-vinylpropanoic acid hydrochloride **4** was obtained from 4-acetoxyazetidin-2-one **1** in 3 steps. The ozonolysis of **4** yielded 3-amino-4-oxopropanoic acid hydrochloride (aspartate 1-semialdehyde hydrochloride **6**). This labile α-aminoaldehyde is isolated in its stable cyclic form, 4-amino-5-hydroxy-4,5-dihydrofuran-2-one hydrochloride **7**. © 1997 Elsevier Science Ltd.

Introduction

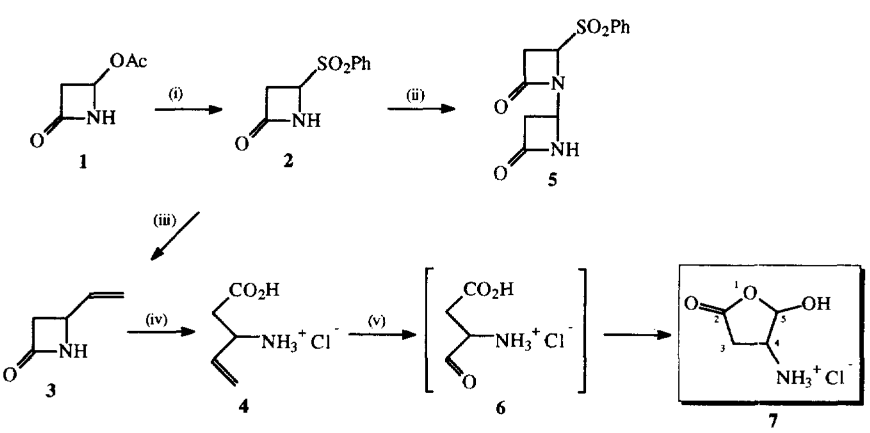
The α-aminoaldehydes of natural amino acids and their derivatives are of great potential interest as enzyme inhibitors[1]. They may also be of value when substituted in place of the C-terminal amino acid in a peptide, as exemplified by naturally occurring inhibitors from plants and microorganisms[2]. Whilst the chemical synthesis of α-aminoaldehydes is straightforward conceptually, their isolation is expected to be problematical due to the strong tendency for them to polymerise, even in acid solution. However reports recently that 4-amino-4-oxobutyric acid hydrochloride (glutamate 1-semialdehyde hydrochloride) may be generated by the ozonolysis of 4-amino-4-vinylbutyric acid (vinyl-GABA)[3] suggested that under some circumstances the aminoaldehyde itself may be possible to isolate, particularly when the side chain carries a functional group capable of forming a ring. We reasoned that aspartate 1-semialdehyde hydrochloride **6**, which had not hitherto been synthesised, could also exist as a cyclic structure and would be accessible by ozonolysis of 3-amino-3-vinypropanoic acid hydrochloride **4** as shown in **Scheme 2**.

Results and Discussion

Our initial strategy (**Scheme 1**) had been to synthesise **4** by selective reduction of 3-amino-4-yne-pentanoic acid[4] **12**, the latter being prepared from **8** via **9**, **10** and **11**. However, unexpectedly, all attempts to hydrolyse **11** failed to yield **12**. The alternative synthetic route to **4** was therefore developed (**Scheme 2**).



Scheme 1 Reagents : (i) PhCHO, (ii) EtMgBr, Me_3SiCl, (iii) BuLi, $BrCH_2CO_2Et$, (iv) 6M HCl, (v) H_2 / Lindlar catalyst

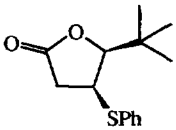


Scheme 2 Reagents : (i) $PhSO_2^-Na^+$, (ii) $(CH_2{=}CH)MgBr$ (1 mole eq.), (iii) $(CH_2{=}CH)MgBr$ (2 mole eq.) (iv) 2M HCl, 60°C, (v) Ozone gas, 1M HCl, 0 °C.

4-Acetoxyazetidin-2-one **1** was added to 1 mole equivalent of sodium benzenesulphinate in water. After stirring the mixture for 24 hours at 45°C, snow-white crystals of 4-phenysulphonylazetidin-2-one **2** were obtained in 32% yield. The crystals were dissolved in THF and 2 mole equivalents of vinylmagnesium bromide in THF were added at -78 °C under nitrogen. The reaction mixture was stirred at this temperature for 20 minutes, then at 0 °C for 30 minutes and finally at room temperature for 1 hour. After work-up, a yellow oil was obtained in 86% yield. Pure, colourless 4-vinylazetidin-2-one **3** was then obtained by distillation at ca. 100 °C and 0.03 mmHg (lit.[6] b.p. = 67 - 68 °C, 0.3 mmHg). The 4-vinylazetidin-2-one **3** was dissolved in 2M HCl and heated at 60 °C for 4 hours, the solvent was then evaporated and the oil was washed with ether. Crystals of 3-amino-3-vinylpropanoic acid hydrochloride **4** were obtained in quantitative yield, m.p. = 119.5 - 120.5 °C. The yield of **3** was, as expected, very sensitive to the amount of vinylmagnesium bromide used. Thus when 1 mole equivalent of vinylmagnesium bromide was added to the 4-phenysulphonylazetidin-2-one **2** under similar conditions, the novel, but unwanted diastereomeric product **5** was obtained in 58.6% yield. No attempts to separate the diastereomers were made.

The synthesis of 3-amino-4-oxopropanoic acid hydrochloride **6** (aspartate 1-semialdehyde hydrochloride) was carried out as follows. 3-Amino-3-vinylpropanoic acid hydrochloride **4** was dissolved in 2M HCl and cooled in an ice bath. Ozone gas in slight excess was passed into the solution and the progress of the reaction was followed by 250 MHz ^1H nmr. The ozonide hydrolysed during the reaction and the product was purified using a Dowex 50W-X8 column under nitrogen in 0.05M HCl. The column was then eluted with 0.5M HCl. A meringue-like, hygroscopic, white solid was obtained on lyophilisation (0.08g, 79% yield) which gave a single ninhydrin positive spot ($R_f = 0.49$) after TLC on silca in n-BuOH:AcOH:H_2O (60:20:20 v/v/v).

The following properties provide strong evidence that the structure of the product of ozonolysis of 4 is the cyclic form of aspartate 1-semialdehyde, 4-amino-5-hydroxy-4,5-dihydrofuran-2-one hydrochloride (HAD). The stretching frequency of the product at 1778 cm^{-1} in the IR spectrum strongly suggests a cyclic, dihydrofuranone structure. The pattern of the ^1H nmr signals exhibited by the geminal pair of protons at the C-3 position indicate that they are non equivalent and are coupled to each other with a large (17.5 Hz) coupling constant. Each is coupled to the proton at the adjacent asymmetric centre with values of 5 Hz and 8.75 Hz. Such behaviour is diagnostic of a restricted conformation expected for a dihydrofuranone ring system. Further evidence in support of this proposal comes from a ^1H - ^1H 2D NOESY experiment (**Figure 1a and 1b**) in which a positive effect was observed between H_4 and H_5. This observation, taken together with the coupling constant data, suggests that the -OH and -NH_3^+ groups are *cis* to one another. The related dihydrofuranone, 4-(phenylthio)-5-*tert*-butyl-4,5-dihydrofuran-2-one **13**[7] exhibits similar nmr chemical shifts and coupling constants to that of **7** and the X-ray structure of **13** confirms it as a 5-membered ring system with the substituents at the 4- and 5-positions, also *cis* to one another. The molecular mass of HAD is 118.1 from the electrospray mass spectroscopy (1 ml acetonitrile/water (1:1) and 1% acetic acid) which corresponds to the cyclic structure of the aspartate 1-semialdehyde, or the free aminoaldehyde, the latter being unlikely under the conditions used.



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Conclusions

From the above data we conclude therefore that the structure of the product of ozonolysis of **4** is the cyclic form of aspartate 1-semialdehyde, 4-amino-5-hydroxy-4,5-dihydrofuran-2-one hydrochloride (HAD) **7**. The formation of a cyclic structure between an aldehydic and a carboxylic acid group is not uncommon. For instance, 4-oxobutanoic acid exists in equilibrium with the cyclic form, 5-hydroxy-4,5-dihydro-2(3H)-furanone (succinate semialdehyde)[8]. The ^1H nmr spectrum[9] shows the open and cyclic form are in equilibrium, depending strongly on the nature of solvents.

Whilst ozonolysis of α-vinylamines, such as **4**, would seem to be a suitably mild method for the generation of the related α-aminoaldehydes, only those capable of forming a ring structure are likely to be stable enough to be isolated. Thus, the unexpected stability of glutamate 1-semialdehyde hydrochloride, an intermediate in tetrapyrrole biosynthesis, may be also be explained by its existence as the cyclic form, 5-amino-6-hydroxy-3,4,5,6-tetrahydropyran-2-one (HAT), although the hydrated aldehyde has also been proposed as an alternative possibility[10].

Since 3-amino-3-vinylpropanoic acid and the higher homologue, 4-amino-4-vinylbutyric acid (vinyl-GABA) have been shown to act as inhibitory neurotransmitters in the mammalian central nervous system[1] by suppressing the function of pyridoxal-5'-phosphate-dependent 4-aminobutyrate:2-oxoglutarate aminotransferase, the related α-aminoaldehydes also have the potential to act as inhibitors of this transaminase and other pyridoxal-5'-phosphate dependent enzymes.

Experimental

General. 4-Acetoxyazetidin-2-one, vinylmagnesium bromide in THF and all solvents used were purchased from Aldrich Chem. Co. Dowex 50W-X8 resins were purchased from Sigma Co. and washed with 2M NaOH, 2M HCl and water respectively prior to use. Proton magnetic resonance spectra (^{1}H nmr) were recorded in $CDCl_3$ and d_6-DMSO with tetramethylsilane as internal reference and D_2O with assignment of the DOH peak at δ 4.8 as reference. Spectra were determined either using a Bruker WP-80DS or a Bruker AM250 instrument unless otherwise stated. Mass spectra were measured using a Kratos MS 50RF spectrometer fitted with a DS90 data system. Ozone gas was generated by the Laboratory Ozonator BA023, made by Wallace and Tieman Co. Electrospray mass spectroscopy was performed using a VG BIO-Q machine in the Dyson Perrins Laboratory, Oxford.

4-Phenysulphonylazetidin-2-one (2) : 4-Acetoxyazetidin-2-one **1** (3.7g, 2.9×10^{-2} mole) was added to sodium benzenesulphinate dihydrate (5.74g, 2.93×10^{-2} mole) in water (30 ml). After stirring the mixture for 24 hours at 45°C, snow-white crystals (1.9g, 31.5% yield) were obtained, m.p. = 155.5 - 157°C (lit.[5]m.p. = 156 - 157 °C). δ_H (d_6-DMSO) (80 MHz) : 9.00 (1H, s, broad), 7.88 (5H, m), 5.10 (1H, dd, J = 5, 2 Hz), 2.88 - 3.50 (2H, m).

4-Vinylazetidin-2-one (3) : 4-Phenysulphonylazetidin-2-one **2** (2.03g, 9.62×10^{-3} mole) was dissolved in dried THF (30 ml) and the solution was cooled at -78 °C in nitrogen. 1M Vinylmagnesium bromide in THF (22.2 ml, 2.2×10^{-2} mole) was added slowly through a syringe. The solution changed to a yellow suspension. The reaction mixture was stirred at this temperature for 20 minutes, then at 0 °C for 30 minutes and finally at room temperature for 1 hour. Saturated ammonium chloride was added to the suspension and the solvent was evaporated. $CHCl_3$ was added to the solids and, after filtration and solvent evaporation, a yellow oil (0.8g, 86% yield) was obtained. Pure, colourless 4-vinylazetidin-2-one **3** was then collected by distillation at ca. 100 °C and 0.03 mmHg (lit.[6] b.p. = 67 - 68 °C, 0.3 mmHg). δ_H ($CDCl_3$) (250 MHz) : 6.10 (1H, s, broad), 5.92 (1H, m), 5.32 (1H, ddd, J = 17.5, 1.25 and 1.25 Hz), 5.20 (1H, ddd, J = 10, 1.25 and 1.25 Hz), 4.15 (1H, m), 3.23 (1H, ddd, J = 15, 5 and 2.5 Hz), 2.72 (1H, ddd, J = 15, 2.5 and 1.75)

3-Amino-3-vinylpropanoic acid hydrochloride (4) : 4-Vinylazetidin-2-one **3** (0.1g, 1.03×10^{-3} mole) was dissolved in 2M HCl (8 ml) and heated at 60°C for 4 hours. The HCl was evaporated and the remaining syrup was rinsed twice with ether and dried in vacuo. Light brown crystals (0.14g, 89.6% yield) were obtained, m.p. = 119.5 - 120.5°C. δ_H (D_2O) (250 MHz) : 5.93 (1H, m), 5.45 (2H, m), 4.22 (1H, q, broad), 2.80 - 2.95 (2H, m). MS *m/z* : (M^+ - 1) (115.1, 9.4), (97.1, 11.2), (70.0, 12.2), (56.1, 100).

4-Amino-5-hydroxy-4,5-dihydrofuran-2-one hydrochloride (HAD) (7) : 3-Amino-3-vinylpropanoic acid hydrochloride **4** (0.1g, 6.59×10^{-4} mole) was dissolved in 2M HCl and cooled in an ice bath. Ozone gas in slight excess was passed into the solution and the progress of the reaction was followed by 250 MHz ^{1}H nmr. The ozonide hydrolysed during the reaction and the product was applied to a Dowex 50W-X8 column under nitrogen in 0.05M HCl. The column was then eluted with 0.5M HCl. A meringue-like, hygroscopic, white solid was obtained on lyophilisation (0.08g, 79% yield) which gave a single ninhydrin positive spot (R_f = 0.49) after TLC on silca in n-BuOH:AcOH:H_2O (60:20:20 v/v/v). ν_{max} / cm^{-1} (KBr) 3500 - 3000 (broad -NH_3^+ and -OH stretching), 1778 (dihydrofuranone C=O stretching), 1600 and 1500 (-NH_3^+ bending); δ_H

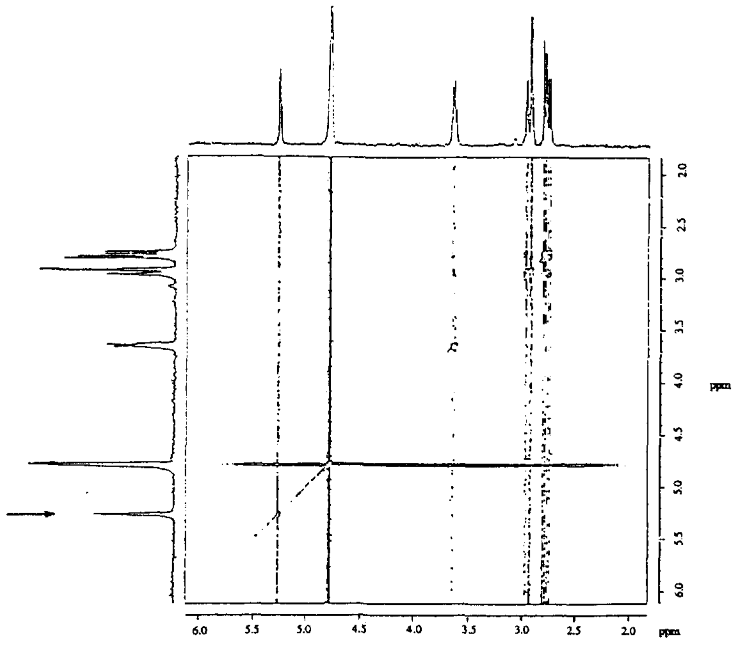

Figure 1a. The ^{1}H-^{1}H 2D NOESY of HAD.

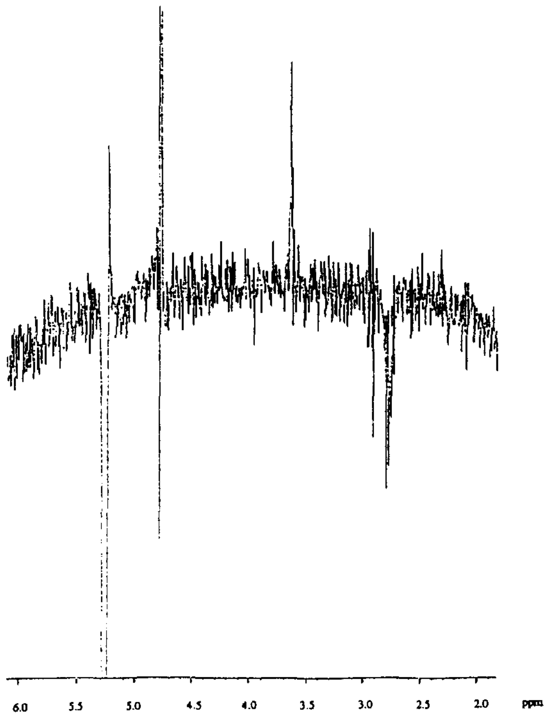

Figure 1b. The NOE from the ^{1}H-^{1}H 2D NOESY data set at the row indicated by the arrow in Figure 1a.

(D_2O) (250 MHz) : 5.27 (1H, d, J = 5 Hz), 3.65 (1H, ddd, J = 5, 5 and 8.75 Hz), 2.95 (1H, dd, J = 5 and 17.5 Hz) and 2.78 (1H, dd, J = 8.75 and 17.5 Hz). δ_C (D_2O) : 176.758 (C=O), 90.536 (-OCHOH), 55.225 (-CHNH$_2$), 35.037 (-CH$_2$-). ES-MS : M^+ (118, 100).

N-(4-Azetidin-2-onyl)-4-phenysulphonylazetidin-2-one (5) : The preparation was same as that of compound **3** except one mole equivalent of vinylmagnesium bromide was added instead of two. After work-up, light yellow crystals (58% yield) were obtained as a mixture of two diastereomers in a ratio of 2.5 : 1. δ_H ($CDCl_3$) (250 MHz) of first diastereomer : 7.60 - 7.95 (5H, m), 6.47 (1H, broad), 5.11 (1H, dd, J = 5 and 2.5 Hz), 4.79 (1H, dd, J = 5 and 2.5 Hz), 3.95 (1H, dd, J = 15 and 2.5 Hz), 2.95 - 3.30 (3H, m). Second diastereomer : 7.60 - 7.95 (5H, m), 6.32 (1H, broad), 5.28 (1H, dd, J = 5 and 2.5 Hz), 4.82 (1H, dd, J = 5 and 2.5 Hz), 3.42 (1H, dd, J = 15 and 2.5 Hz), 2.95 - 3.30 (3H, m). MS *m/z* : M^+ (280.1, 1.8), (139.0, 74.8), (138.0 47.2), (77, 100).

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