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N^2-Hydroxyasparagine[1]

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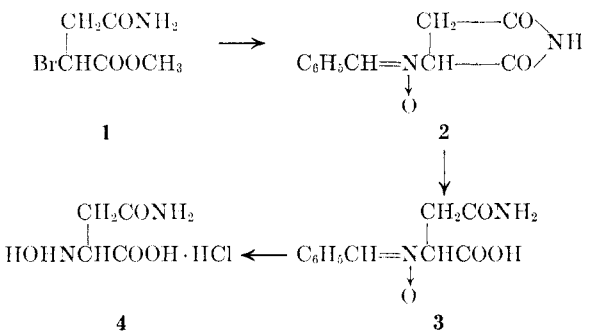
Because of the reported role of asparaginase in certain asparagine-requiring tumors,[2-4] the study of analogs of this amino acid seemed desirable. We have prepared the asparagine analog N^2-hydroxyasparagine through an extension of a general procedure for α-N-hydroxyamino acids published previously.[5,6]

Attempts to prepare the benzal nitrone of succinic acid or of diethyl succinate from their respective α-bromo compounds were unsatisfactory, as was an attempted condensation of β-benzaldoxime with α-bromosuccinamic acid. The reaction succeeds through the methyl ester (**1**) of the latter, in the presence of 2 equiv of sodium methoxide. Condensation with the β-benzaldoxime probably occurs after formation of α-bromosuccinimide to give the imide derivative of the nitrone (**2**).[7] Dilute hydrochloric acid led to cleavage



of the nitrone to 2-hydroxylaminosuccinimide which was identified in the reaction mixture by pmr. It failed to open without further decomposition. Treatment of **2** with only 1 equiv of base (pH 9.5) did not give complete opening of the imide, but maximum yield of open-ring material could be achieved by maintaining the pH at 11–12. This nitrone of 2-aminosuccinamic acid (**3**) proved to be homogenous by thin layer chromatography. Cleavage of the nitrone **3** was then effected by treatment with 1 *N* HCl at room temperature. The compound thus formed, N^2-hydroxyasparagine (**4**), was isolated as its hydrochloride. The product gave a positive Fehling's test and could not be crystallized either as its hydrochloride or the free base. Chromatography showed one major spot. Catalytic hydrogenolysis led to asparagine, with not more than a trace of isoasparagine, as determined by paper chromatography or by electrophoresis. As expected[7] treatment of **2** with concentrated aqueous ammonia gave the diamide, which on treatment with 1 *N* HCl gave 2-hydroxylaminosuccinamide.

Optical activity, retained from L-asparagine to L-α-bromosuccinamic acid,[8] was lost during the formation of the nitrone. Thus, in this reaction series, L-asparagine leads to racemic hydroxylamino compound.

In preliminary assays the presence of 2 molar equiv of DL-N-hydroxyasparagine did not inhibit the hydrolysis of L-asparagine by L-asparaginase. The assay[4] includes recovery of ammonia by aeration at pH 11, and in the absence of the enzyme the analog releases slightly over 1 equiv of ammonia. In the presence of guinea pig serum L-asparaginase or *Escherichia coli* L-asparaginase (EC-2)[4] no additional ammonia was released from the analog. Tests were made at both pH 5.0 (0.1 *M* acetate) and pH 8.5 (0.1 *M* borate) at 37°.

Although it is unstable in strong alkali, we find that N-hydroxyasparagine is considerably more stable than indicated by Emery[9] for a presumed N-hydroxyaspartic acid.

We thank Dr. H. A. Campbell for the assay with asparaginase.

Experimental Section[10]

Benzalnitrone of Succinimide (2).—To a solution of 8.5 g (0.043 mole) of L-α-bromosuccinamic acid prepared from L-asparagine[8] in 20 ml of MeOH was added an excess of CH_2N_2 in 100 ml of dry ether. The reaction mixture was taken to dryness under vacuum and the crude ester was dissolved in 50 ml of EtOH and added to a solution of β-benzaldoxime[11] (5.3 g) and $NaOCH_3$ (2 g of Na) in 100 ml of MeOH. After standing at room temperature overnight, the reaction mixture was concentrated *in vacuo* and the residue was taken up in 200 ml of H_2O. Neutralization with AcOH yielded a precipitate (7 g, 75%) which was recrystallized from MeOH; mp 187–192° dec. *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N.

Benzalnitrone of 2-Aminosuccinamic Acid (3).—To 2.55 g (0.012 mole) of **2** was added 60 ml of 0.2 *N* NaOH plus enough additional alkali to bring the solution to pH 12. After 17 hr at room temperature, the solution was adjusted to pH 3 by the addition of 1 *N* HCl. On standing, glistening plates separated (1.5 g, 53%) which after recrystallization from absolute EtOH melted at 175–176°. Thin layer chromatography on silica gel with BuOH–EtOH–H_2O (3:1:1) and with uv light or Tollens reagent for visualization indicated one component. *Anal.* ($C_{11}H_{12}N_2O_4$) C, H, N.

N^2-Hydroxyasparagine Hydrochloride (4).—To 1.3 g of nitrone **3** was added 10 ml of 1 *N* HCl. The HCl was then removed under vacuum below 30°. The residue was dissolved three times in 10 ml of EtOH and taken to dryness *in vacuo*. The resulting white gum was taken up in 10 ml of EtOH, filtered, and precipitated by the addition of ether. After repeating this twice the residue

(1) This investigation was supported in part by funds from the National Cancer Institute (Grant No. CA 08748).

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was dried to constant weight *in vacuo*. The resulting powder was very hygroscopic, sintered at 50°, and decomposed at 62°. Fehling's solution was reduced and one major spot was noted on thin layer chromatograms on silica gel developed with BuOH–EtOH–H_2O (visualized by Tollens reagent). *Anal.* ($C_4H_8N_2O_4 \cdot HCl$) C, H; N: calcd, 15.18; found, 15.74.

Hydrogenolysis of 4.—A solution of 184 mg of N-hydroxy compound (**4**) as its hydrochloride in 25 ml of EtOH was hydrogenated at atmospheric pressure with PtO_2 (50 mg). After 6 hr the theoretical uptake was complete, the catalyst was removed, and the filtrate was taken to dryness. The product, dissolved in a few milliliters of H_2O, gave a negative Fehling's test. Electrophoresis performed with authentic samples of isoasparagine, asparagine, and aspartic acid at pH 3.5 (acetate buffer, 500 v, 4 hr) with ninhydrin as an indicator, or paper chromatography with 88% phenol, showed the product to contain but traces of isoasparagine and aspartic acid. The major component, DL-asparagine, precipitated on adjusting the solution to pH 3, had essentially zero rotation, and contained no isoasparagine.

Benzalnitrone of Succinamide.—A solution of 2 g of **2** in 30 ml of 28% aqueous NH_3 was allowed to stand at room temperature for 24 hr. The diamide precipitated (600 mg, 28%) and was collected and recrystallized from 75% EtOH. It melted at 204–205° dec. *Anal.* ($C_{11}H_{13}N_3O_3$) C, H, N.

2-Hydroxylaminosuccinamide Hydrochloride.—A solution of 350 mg of the above nitrone in 10 ml of 1 *N* HCl was concentrated *in vacuo* keeping the bath temperature below 30°. The resulting precipitate was taken up twice in EtOH and precipitated by the addition of ether. The white powder, dried *in vacuo* to constant weight, decomposed at about 80°. Chromatography with BuOH–EtOH–H_2O indicated one component with Tollens reagent at R_f 0.3. *Anal.* ($C_4H_9N_3O_3 \cdot HCl$) C, H, N.

Some 3-Methyl-2-butenylaminopurines and -pyrimidines[1]

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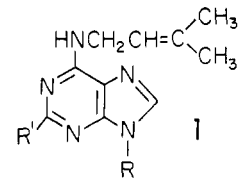
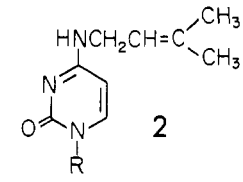
6-(3-Methyl-2-butenylamino)-9-β-D-ribofuranosylpurine has been recently reported as a minor component of yeast and liver soluble ribonucleic acid[2] and of yeast serine transfer ribonucleic acid.[3,4] 6-(3-Methyl-2-butenylamino)purine has been found in extracts of *Corynebacterium fascians*.[5] Both compounds have been synthesized and exhibit a high level of cytokinin activity.[2,6] It was therefore of interest to synthesize other 3-methyl-2-butenylamino pyrimidines and purines and to test them for biological activity.

In all cases the syntheses were carried out by refluxing an excess of γ,γ-dimethylallylamine with the appropriate mercapto or alkylmercaptopurine or -pyrimidine according to the method of Elion, *et al.*[7] The reactions were followed spectrophotometrically in ethanol and were terminated when the absorption maximum of the



R	R'
a. H	NH_2
b. ribofuranosyl	NH_2
c. H	OH



R
a. H
b. ribofuranosyl

starting material (297–315 mμ) was no longer present. Proof of structure of the products **1a–c** and **2a, b** was shown by the similarity of their ultraviolet absorption spectra to the purine N^6- or pyrimidine N^4-monoalkylated parent compounds (Table I). That the double bond in the side chain is in the position shown is demonstrated by the nmr methyl signals at δ 1.75 (DMSO-d_6, TMS).[2,8]

In the leaf senescence test[9] all of the compounds (**1** and **2**) showed about one-tenth the activity reported for 6-furfurylaminopurine (kinetin).[10] None of these compounds exhibited any activity against mouse leukemia L1210 *in vivo*.[11] Compounds **2a** and **2b** were also inactive against Burkitt's cell cultures.[11a] The cytidine analog **2b** was not deaminated by human liver or mouse kidney enzyme systems.[11a]

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are corrected. Spectra were determined using a Cary Model 15 spectrophotometer. Analyses were done by Spang Microanalytical Laboratory, Ann Arbor, Mich., and Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

2-Amino-6-(3-methyl-2-butenylamino)purine (1a).—2-Amino-6-methylthiopurine[12] (1.25 g, 6.9 mmoles) and γ,γ-dimethylallylamine[13] (5.0 g, 59 mmoles) were refluxed for 30 hr. A precipitate which formed on cooling was washed with petroleum ether (30–60°) to remove the excess amine and was dissolved in 95% EtOH. After the addition of concentrated HCl, 1.18 g (67%) of **1a** hydrochloride was obtained. A sample for analysis was recrystallized from EtOH after treating with charcoal; mp 250–251° dec. *Anal.* ($C_{10}H_{14}N_6 \cdot HCl$) C, H, N, Cl.

2-Amino-6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (1b).—6-Ethylthioguanosine[14] (2.0 g, 6.1 mmoles) and γ,γ-dimethylallylamine (6.0 g, 71 mmoles) were refluxed for 60 hr. After the addition of petroleum ether (30–60°), a dark oil separated. The oil was triturated several times with petroleum ether to remove any excess amine and was then dissolved in 95% EtOH, treated with charcoal, and filtered. The crystals which formed were washed with cold EtOH and dried (yield 1.31 g, 48%). A sample for analysis was recrystallized from EtOH, mp 93–97°. *Anal.* ($C_{15}H_{22}N_6O_4 \cdot EtOH$) C, H, N.

2-Hydroxy-6-(3-methyl-2-butenylamino)purine (1c).—2-Hydroxy-6-methylthiopurine[15] (0.42 g, 2.3 mmoles), γ,γ-dimethylallylamine (4.0 g, 47 mmoles), and EtOH (5.0 ml) were refluxed for 90 min. The crystals which formed on cooling were washed with EtOH, dried, dissolved in 1 *N* NaOH, and reprecipitated

(1) (a) This investigation was supported in part by National Cancer Institute Grant CA 08748. (b) The side chains of these compounds have also been referred to as γ,γ-dimethylallylamino or Δ²-isopentenyl groups.

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