ter, sturdy needles started to crystallize within a few minutes. The crystals were filtered after 3 days and amounted to 2.8 g. (63.6%). By recrystallization from water, XI was obtained in the form of white prisms melting at 216–217° (with bubbling). The infrared spectrum shows salt formation between carboxylic and amino groups at 1350, 1620 and 3120 cm.$^{-1}$.

Anal. Calcd. for $C_6H_{12}N_6O_4$: C, 27.27; H, 5.49; N, 38.17. Found: C, 27.03; H, 5.35; N, 38.39.

Infrared spectra were determined on a Perkin–Elmer double beam spectrophotometer, model 137, using solid samples dispersed in potassium bromide.

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A Convenient Preparation of 5-Amino-4-imidazolecarboxamide Riboside. Ring Opening of N^1-*p*-Toluenesulfonyl-inosine

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The nucleic acid precursor, 5-amino-4-imidazolecarboxamide riboside is now made readily available from inosine (hypoxanthine riboside) by a new degradative reaction in which a purine riboside is converted to an imidazole riboside. The N^1-tosyl derivative of inosine is easily prepared and hydrolyzes in alkali to 5-amino-4-imidazole-N-(tosyl)-carboxamide riboside (II). Hydrazinolysis removes *p*-toluenesulfonamide from this imide and provides the imidazole carboxhydrazide III which undergoes smooth hydrogenolysis to the amide IV.

Recently it was shown that, although inosine is very stable to alkali, 1-benzylinosine undergoes an alkaline hydrolysis of the purine ring to an imidazole nucleoside.[1] This observation was made use of in the preparation of aminoimidazolecarboxamide riboside from inosine. Difficulty in removing the benzyl group limited the value of this method and a different labilizing group was sought. Since the ring-opening reaction apparently depended merely on the replacement of the ionizable hydrogen at N-1 of the purine ring, the chemical nature of the group used for the purpose did not appear to be important, providing that it had alkaline stability greater than the ring. The sulfonyl group suggested itself since the sulfonamide bond, if it formed in this case, would probably have its characteristic high stability. An eventual removal of the sulfonyl group together with the ring nitrogen was visualized as more promising than the earlier difficult debenzylation.

Sulfonamide derivatives of purines of the type shown in I do not appear to have been prepared before. 1-*p*-(Toluenesulfonyl)-inosine was obtained as the triacetate in good yield when inosine triacetate, converted to its sodium salt by means of sodium hydride in dimethylformamide, was treated with *p*-toluenesulfonyl chloride. The structure of the product is indicated by the following reactions. When the nucleoside was heated with alkali, formation of a primary aromatic amine was soon detectable by diazotization and coupling.[2] This test permitted determination of the optimal time for complete reaction. The product, 4-amino-5-imidazole-N-(*p*-toluenesulfonyl)-carboxamide ribofuranoside (II) was readily crystallized from the reaction mixture and, in addition to the aromatic amino group, had the expected acidic properties, *viz.*, a pK_a of 6.3 (in 35% aqueous dimethylformamide).[3] Attempts were then made to split the imide structure of II in order to remove the *p*-toluenesulfonamide group. The substance was not altered by prolonged treatment with hot alkali. This stability toward alkali is in contrast to the cleavage of saccharin to *o*-carboxybenzenesulfonamide.[4] However, although saccharin is the most familiar example of an imide such as II, it contains this grouping in a five-membered ring where it may be somewhat strained and therefore more labile than an acyclic example. Ammonolysis with concentrated ammonium hydroxide in sealed tubes could be achieved only at elevated temperatures (*ca.* 160°) and was not considered promising as a means of proceeding directly to the amide due to accompanying decomposition. The cleanest cleavage of the N-sulfonylcarboxamide (II) was accomplished by hydrazinolysis to *p*-toluene-sulfonamide and the hydrazide of 5-amino-4-imidazolecarboxylic acid riboside (III). The hydrazide was reduced to the amide by means of Raney nickel in ethanol[5] which provided the desired riboside IV in satisfactory yield.

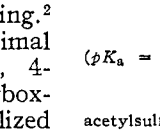
The method described here is very convenient for the laboratory preparation of 5-amino-4-imidazolecarboxamide riboside in gram quantities.[6] The extension to related ribosides and ribotides of biosynthetic importance is under study.

Experimental[7]

1-(*p*-Toluenesulfonyl)-inosine Triacetate.—Sodium hydride (1.5 g. of the 50% dispersion in mineral oil, Metal Hydrides Corp.) was added to dimethylformamide (100 ml.) in

(1) E. Shaw, THIS JOURNAL, **80**, 3899 (1958).

(2) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

(3) Measurements of the acid strength of strictly comparable models have not been reported. However, derivatives of sulfanilamide (pK_a = 10.43) which contain the  grouping such as N^1-acetylsulfanilamide (pK_a = 5.38) suggest that the observed value for II is a reasonable one for the structure. The data are from P. H. Bell and R. O. Roblin, THIS JOURNAL, **64**, 2905 (1942).

(4) C. Fahlberg and R. List, *Ber.*, **21**, 242 (1888).

(5) S. Akabori and K. Narita, *Proc. Japan Acad.*, **29**, 264 (1953); *cf. C. A.*, **49**, 864 (1955); C. Ainsworth, THIS JOURNAL, **76**, 5774 (1954).

(6) Preliminary account of a synthesis of this nucleoside starting with methyl 5-nitro-4-imidazolecarboxylate and ribofuranose tribenzoate has appeared: J. Baddiley, J. G. Buchanan and J. Stewart, *Proc. Chem. Soc.*, 149 (1957).

(7) Melting points were taken on a Fisher block and are uncorrected. Microanalyses were performed by the Scandinavian Microanalytical Laboratory, Copenhagen.

a dry flask protected with a drying tube. When the hydride was uniformly suspended, powdered, dry inosine triacetate[8] (9.0 g.) was introduced. Within a few minutes hydrogen evolution ceased and solution took place. Finely divided *p*-toluenesulfonyl chloride (4.5 g.) was added. The mixture was maintained at 60° for one hour, then left overnight at room temperature. After addition of a few ml. of absolute ethanol to decompose unreacted sodium hydride, the mixture was neutralized with acetic acid, if necessary, and concentrated to a sirup under reduced pressure while heated below 50° in a water-bath. The viscous residue was stirred with water (150 ml.), whereupon the crude product granulated, was filtered with suction, washed with water, and dried. To remove any unreacted inosine triacetate that may have remained in the product, the latter was stirred for a few minutes with cold 0.5 *N* NaOH (100 ml.), refiltered, washed and dried. This insoluble material was used for ring opening as described below without further purification. With the assumption that no deacetylation took place during the process, the yield, 10.5–11.5 g., was calculated as about 90% of theory.

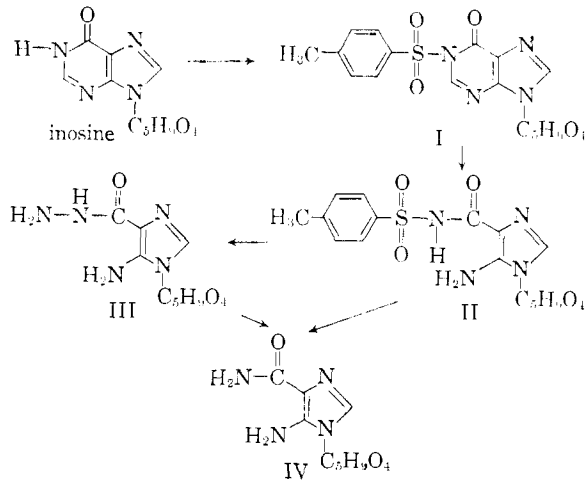
5-Amino-4-imidazole-N-(*p*-toluenesulfonyl)-carboxamide Riboside (II).—1-(*p*-Toluenesulfonyl)-inosine triacetate (5.0 g.) was refluxed for 5.5 hours in ethanol (500 ml.) and 5 *N* sodium hydroxide (15 ml.). The solution was taken to dryness under reduced pressure and, after addition of water, the concentration was repeated. The residue was then dissolved in water (50 ml.) and filtered through wet filter paper to remove traces of mineral oil carried through from the initial sodium hydride condensation. When the filtrate was brought to *p*H 5 with acetic acid, crystallization of the product began. The suspension was left at 4° overnight, filtered with suction, washed with water and dried *in vacuo* to yield 2.55 g. (68%), m.p. 207–209°.

At *p*H 4.5, the product exhibited a single maximum at 282 mμ, ε 15,500, a minimum near 250 mμ, and high end absorption due to the toluenesulfonyl grouping.

In carrying out the Bratton-Marshall test[2] on II it was found that the resultant pigment was not very soluble in water. For colorimetric analysis, an equal volume of ethanol was added ten minutes after the coupling reaction. A linear relation of color produced (λ_{max} 565 mμ) to concentration of amine (final values 0–20 μg./ml.) was obtained with this modification.

Anal. Calcd. for $C_{16}H_{20}O_7N_4S$: C, 46.59; H, 4.89; N, 13.59; S, 7.77. Found: C, 46.01; H, 5.20; N, 13.29; S, 8.04.

5-Amino-4-imidazolecarboxhydrazide Riboside.—5-Amino-4-imidazole-N-(*p*-toluenesulfonyl)-carboxamide riboside (3.6 g.) was refluxed for ten hours with anhydrous hydrazine (75 ml.). The solution was taken to a sirup under reduced pressure and the residue left in an evacuated desiccator over concentrated sulfuric acid to remove hydrazine completely. Water (10 ml.) was stirred into the mass followed by acetic acid to bring the *p*H to 7. The insoluble material removed by filtration at this point was a part of the *p*-toluenesulfonamide formed in the reaction. The remainder of this by-product and any unreacted starting material were removed now by taking advantage of their negative charge in alkali due to the sulfonamide grouping. For this purpose, the filtrate was diluted to 300 ml. and maintained at *p*H 8 by addition of ammonium hydroxide. Dowex-2 chloride was



added portionwise with stirring until inspection of filtered samples revealed disappearance of high absorption in the 230 mμ region and adjustment of the 270/290 mμ optical density ratio to 3.6 from lower values. Addition of more resin than necessary to accomplish this was avoided to minimize losses due to absorption of the product. The filtrate was taken to a small volume under reduced pressure and allowed to crystallize slowly. The resultant mass was thinned with 50% ethanol for filtration. There was obtained 1.4 g. (59%), m.p. 186–187°.

Anal. Calcd. for $C_9H_{15}O_5N_5$: C, 39.56; H, 5.53; N, 25.64. Found: C, 39.65; H, 5.72; N, 25.02.

5-Amino-4-imidazolecarboxamide Riboside.—5-Amino-4-imidazolecarboxhydrazide riboside (1.0 g.) was dissolved in 95% ethanol (60 ml.) and water (15 ml.) and refluxed for two hours with Raney nickel catalyst[9] (5.0 g. wet weight of Raney nickel in water). The filtrate, which gave a negative test for the hydrazide group when tested with ammoniacal silver, was concentrated under reduced pressure. The residue crystallized completely, 0.81 g. (85%); it was recrystallized from water (4 ml.), left at 4° overnight, thinned with ethanol, and filtered, yielding 0.52 g. (55%) of the nucleoside identical with an authentic sample[10] when compared by paper chromatography, ultraviolet and infrared spectroscopy.

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(8) H. Bredereck, *Ber.*, **80**, 401 (1947).

(9) Raney catalyst in water was obtained from the Raney Catalyst Co., Chattanooga, Tennessee.

(10) G. R. Greenberg and E. L. Spilman, *J. Biol. Chem.*, **219**, 411 (1956).