companies the displacement of the chlorosulfite group by chloride in the decomposition of secondary alkyl chlorosulfites,[3] we have studied the kinetics and products from the decomposition of an extensively deuterated 2-pentyl chlorosulfite in dioxane. Table I shows the results of these experiments both on the undeuterated compound and on a sample of chlorosulfite made from 2-pentanol in which 86% of the hydrogens on the 1- and 3-positions were replaced by deuterium by exchange of the corresponding ketone.

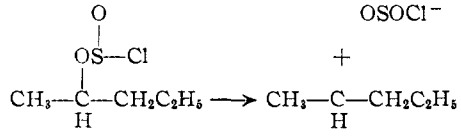
TABLE I

FIRST ORDER RATE CONSTANTS AND PRODUCT YIELDS IN THE DECOMPOSITION OF 2-PENTYL CHLOROSULFITE IN DIOXANE

	Protium comp.	Deuterium comp.
$k \times 10^4$ sec.$^{-1}$ at 61.5°	2.2 ± 0.1	1.6 ± 0.2
$k \times 10^4$ sec.$^{-1}$ at 77.5°	9.8 ± .1	6.2 ± .2
$C_5H_{11}Cl$ yield, 61.5°	51 ± 1%	51 ± 1%
$C_5H_{11}Cl$ yield, 77.5°	45.0 ± 1%	45.6 ± 1%

We also found that the yield of hydrogen chloride was not markedly altered by the introduction of deuterium, but the data were not well reproducible, due to analytical difficulties; however, it is clear that alkyl chloride, olefin, hydrogen chloride and sulfur dioxide account for substantially all of the chlorosulfite. Combustion of 2-pentene isolated from the runs at 62.5° showed that 85% of the hydrogen at the 1- and 3-positions was substituted by deuterium, within experimental error the same as the starting alcohol.

Since the product ratios are not significantly altered, it is apparent that the rate of chloride production is appreciably reduced, although all bonds to hydrogen in the chlorosulfite are intact in the product. The mechanism of this reaction (neglecting solvent effects) appears to be a rate-determining ionization to an undissociated ion pair,[4] which can for simplicity be written



The chloride then results in attack of chloride ion at the 2-position and the olefin from attack of some base on the hydrogens at the 3-position. The source of this unusual isotope effect appears to be the weakening of the bonds to the hydrogen in the 1- and 3-positions in the carbonium ion and the transition state due to hyperconjugation structures with no bond to hydrogen. The change in the force constants of these bonds then produces a different change in the zero-point energies of vibration of the protium and deuterium compounds during the reaction, and the usual rate difference results. The failure to observe significant isotope fractionation in olefin formation can also be attributed to the great weakness of these bonds at the stage when the olefin is formed.

We believe that this type of isotope effect can be used to detect hyperconjugation in the transition state and hence may be used as a measure of the extent of electron deficiency on carbon in the transition state of a displacement reaction. This has so far been determined only by very indirect and dubiously reliable methods.

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ISOLATION OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE RIBOSIDE FROM THE CULTURE MEDIUM OF SULFONAMIDE-INHIBITED *ESCHERICHIA COLI*

Sirs:

4-Amino-5-imidazolecarboxamide which has been isolated from sulfonamide-inhibited cultures of *Escherichia coli*[1,2] has been considered as a possible intermediate compound in purine biosynthesis,[2,3,4] but several lines of evidence have suggested that instead its riboside or ribotide is an intermediate.[5,6,7,8] If it is considered that ring closure of the carboxamide occurs at the ribotide level,[5,7] then it might be expected that in the presence of sulfonamide[8] the carboxamide ribotide as well as the riboside and free base could accumulate. 4-Amino-5-imidazolecarboxamide riboside is shown by the present studies to be the major carboxamide component in young cultures of sulfadiazine-inhibited *E. coli*.

A procedure for isolation of the riboside on a small scale is given. *E. coli*, strain B from a nutrient agar slant, is inoculated into a 24 × 200 mm. tube containing 10 ml. of glucose, salts and NH_4Cl medium.[9] After 16 hr. of incubation at 37° the culture is inoculated into a 1-liter erlenmeyer flask containing 250 ml. of the same medium plus 2.8 mg. of sulfadiazine and 7.5 mg. of glycine. A stationary incubation is carried out for 11 hr. at 37°. The cells are removed by centrifugation in the cold. Extracts of the boiled cells contain little carboxamide. Approximately 100 μM. of diazotizable, non-acetylatable amine[3] is formed per liter of culture medium. The medium is lyophilized to dryness, taken up in a minimum of water, and the sirupy mixture is deposited in a continuous narrow line on 5 sheets of 18.5 × 22.5″ Whatman No. 1 filter paper for chromatography. These are chromatographed with 80% *n*-propanol in H_2O (v./v.). Several components can be visualized with the ultraviolet lamp (Mineralite). Some of these can be detected as diazotizable amines.[3] More than 90% of the diazotizable non-acetylatable amine re-

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sides in a discrete band, the center of which varies from R_f 0.33 to 0.40. Another band at approximately R_f 0.57 corresponds to the free base and the sulfadiazine is at 0.86. The 0.40 fraction is eluted with water and after lyophilizing rechromatographed on 2 sheets of filter paper with 60% *n*-propanol in water in the presence of a beaker of 1 *M* NH_4OH. The compound shows an R_f of approximately 0.65. A third chromatographing of the compound for 24 hr. at room temperature with the organic phase of a mixture of *n*-butanol, acetic acid and water in the volume proportions of 4:1:5 allowing the solvent to run off the paper removes carbohydrate impurities. Assigning a migration value of 1.0 to D-ribose, the pentoside moves to 1.19. The resulting compound remains as a single orcinol-reacting, diazotizable, non-acetylatable amine through chromatography with a wide variety of solvents. In seven solvents the movement of the pentoside on paper is slower than that of the carboxamide. The movement of the compound on paper and its analysis precludes its containing a phosphate ester.

This carboxamide compound shows a maximum absorption at about 267 mμ at *p*H 7 with a curve closely similar to that of the free base. Analysis of the imidazolecarboxamide and pentose moieties of the riboside in terms of ultraviolet absorption maximum, diazotizable amine[8] and orcinol reaction shows these relative molar ratios

Density 267 mμ[a]	Diazotizable amine[a]	Pentose[b]
1.17	1.00	1.00

[a] Standard: 4-NH_4-5-imidazolecarboxamide based on $\epsilon_{267\ m\mu}$ of 1.27×10^4. [b] Method of W. Mejbaum,[10] heating 40 min.; standard, recrystallized D-ribose.[11]

The variation from 1:1:1 may be ascribed in part to the probable difference between the ultraviolet extinction coefficients of the free base and the riboside.

Hydrolysis of carboxamide riboside with 0.5 *N* hydrochloric acid 30 min. at 100° liberates the free carboxamide as demonstrated by chromatographing the hydrolysate with four solvents and comparing the R_f values, ultraviolet absorption and diazotization reaction of the base which is formed with that of the authentic imidazolecarboxamide. Acid hydrolysis liberates an aldose-reacting sugar which tentatively may be considered to be ribose. Using paper chromatography with four solvents, the sugar corresponds to D-ribose rather than arabinose, lyxose, or xylose and shows a pink color with aniline phthalate reagent.[12] The absorption spectrum of the Bial orcinol reaction product of the riboside corresponds closely to that of the aldopentoses. The nature or position of the riboside linkage is not certainly defined. Since dilute acid effects hydrolysis of the glycosidic linkage and since the ultraviolet absorption spectrum of the riboside is similar to that of the free base, it is assumed that the ribose is attached to the nitrogen which would correspond to the number 9 position in the purine.[13] 4-Amino-5-imidazolecarboxamide riboside is unstable on standing on paper, being converted to a red compound. Thus the yield tends to be low (approximately 30%) but is dependent on the care taken. It is apparent that the earlier method of isolating the carboxamide[1,2] may have caused acid hydrolysis of its ribose derivatives.

Recently Ben-Ishai and co-workers[14] tentatively identified the desoxyriboside of carboxamide as the product of the incubation of the carboxamide with *E. coli* suspensions, and MacNutt[15] employing *Lactobacillus helveticus* extracts has presented evidence for transglycosidation from thymidine to imidazolecarboxamide to form the carboxamide desoxyriboside. In the present studies the riboside is synthesized by a *de novo* pathway as is shown by the fact that glycine-1-C^{14} is converted to the carboxamide moiety of the riboside with little dilution.

The demonstration of the occurrence of this compound as the major component of the diazotizable compounds in these young cultures does not necessarily constitute evidence that *per se* it is an intermediate reactant in purine nucleotide synthesis. It may in fact represent a degradation product perhaps from the ribotide. Details of experiments regarding the formation of the carboxamide compounds and the large scale isolation of the riboside will be presented elsewhere.

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(17) I wish to thank Mrs. Hale Bumpus for her valuable assistance.

THE PHOSPHORUS IN PEPSIN AND PEPSINOGEN

Sir:

If the molecular weights of pepsin and pepsinogen are 35,000 and 38,000, respectively,[1] each of these molecules contains one atom of phosphorus.[2,3] Since it has been shown that ovalbumin and α-casein are dephosphorylated by certain phosphatases from mammalian tissue, and from potato,[4,5,6] it was of interest to study the action of these enzymes on pepsin and its precursor. If dephosphorylation occurs, the influence of this reaction on the proteolytic activity of pepsin and on the pepsinogen-pepsin transformation becomes of considerable interest.

The pepsinogen used in this work was kindly supplied by Dr. Roger M. Herriott of the School of Hygiene and Public Health of Johns Hopkins University, while the crystalline pepsin was a Worthing-

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