of pig heart[6] (Table II). Cysteine used in this experiment was later found to be unnecessary. This enzyme contains bound protogen and cocarboxylase. Addition of cocarboxylase, or of diphosphopyridine nucleotide (DPN), coenzyme A (CoA) and cysteine did not change the rate of incorporation. C^{14} labeled formate and succinate were not incorporated with or without CoA and DPN.

TABLE II

INCORPORATION OF LABELED CARBON DIOXIDE INTO α-KETOGLUTARATE

The components of the system were α-ketoglutarate (10 μmoles), cysteine (10 μmoles), $NaHC^{14}O_3$ (1.8×10^5 cts./min.) and α-ketoglutaric oxidase in a total volume of 0.5 ml. at *p*H 7.0; incubated at 37° for 15 min.

Oxidase units	Cts./min./μ mole
5	15
13	40
25	86
50	198
75	256
75 (boiled)	0

The keto acids were purified for counting by (a) partition chromatography of the 2,4-dinitrophenylhydrazones on silica gel column[7] and (b) recrystallization of the 2,4-dinitrophenylhydrazones (with carrier) to constant specific activity. Both methods showed the same specific activity.

(6) D. R. Sanadi and J. W. Littlefield, XIIth International Congress of Pure and Applied Chemistry, New York, Sept., 1951; *J. Biol. Chem.*, in press.

(7) D. O. Brummond, "The oxidation of organic acids by mitochondria from plants," M.S. Thesis, University of Wisconsin, 1952.

INSTITUTE FOR ENZYME RESEARCH
UNIVERSITY OF WISCONSIN MORRIS GOLDBERG
MADISON, WIS. D. R. SANADI

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THE CONFIGURATION OF UNDISSOCIATED SULFAMIC ACID

Sir:

In a recent paper on the ionization constant of sulfamic acid,[1] the statement is made that "Although the acid occurs in its crystals as a dipolar ion, $^+NH_3SO_3^-$, the un-ionized acid in aqueous solution is largely in the form of the neutral molecule, NH_2SO_3H." As a basis for this statement, reference is made to a paper by Baumgarten.[2] This same concept of the configuration of undissociated sulfamic acid in aqueous solution is expressed by Kanda and King,[3] apparently on the same basis.

It is the purpose of this communication to point out (1) that Baumgarten's paper gives no evidence for this configuration (in fact, the statement made by Baumgarten which has been interpreted[4] as claiming the normal configuration for the undissociated molecules probably was not meant to imply this at all) and further, (2) that there appears to be no compelling reason to expect the undissociated molecules in aqueous solution to be other than the dipolar ion form, which clearly is the configuration in the crystals,[3] although it is not at present possible to determine unambiguously the configuration in solution.

In order to determine, in the absence of direct molecular structure evidence, which of the two configurations is correct, one can only attempt to decide which would be the weaker acid. Then, since the ion produced by either configuration is the same, the addition of hydrogen ions to this sulfamate ion to form the undissociated molecules must necessarily form this more weakly acidic configuration.

Unfortunately, it does not seem possible to calculate expected acid strengths for either configuration with sufficient accuracy to permit an unequivocal answer. Using the semi-empirical method of Branch and Calvin,[5] which is known to be not too satisfactory for acids of this strength, one may estimate for the dipolar ion form $pK(^+NH_3SO_3^-) = pK(NH_4^+) + \log 4/3 + \{pK(HOSO_3^-) - pK(HOH) + \log 2 \times 4\} = 9.3 + 0.1 + \{1.7 - 16.0 + 0.09\} = -4.0$, or from the inductive constants given by Branch and Calvin, $pK(^+NH_3SO_3^-) = pK(NH_4) - I_s - 2I_+ - (3/2.8)I_0 - (3/2.8)I_- + \log 4/3 = 9.3 - 3.4 - 2(12.3) - (3/2.8)(4) - (3/2.8)(-12.3) + 0.1 = -9.7$. Similarly for the neutral molecule form $pK(NH_2SO_3H) = pK(H_2O) - I_s - 2I_+ - (2/2.8)I_0 - (2/2.8)I_- - (1/2.8)I_N + \log 3 = 16.0 - 3.4 - 2(12.3) - (2/2.8)(4) - (2/2.8)(-12.3) - (1/2.8)(1.3) + 0.5 = -6.2$ or $pK(NH_2SO_3H) = pK(HSO_4^-) + \log 3/4 + (1/2.8)\cdot I_0 + (1/2.8)I_- - (1/2.8)I_N = 1.7 - 0.1 + (1/2.8)(4) + (1/2.8)(-12.3) - (1/2.8)(1.3) = -1.9$. While these values appear to be slightly in favor of the neutral molecule form, it is obvious that the difference is not sufficient to make any real decision possible. The fact that the S–N bond distance in the sulfamate ion is 1.60 Å.,[6] indicating considerable double bond character, is in the direction to hinder the attachment of the proton to the nitrogen atom, again does not compel the assumption of the neutral molecule form for the acid.

Thus, since there seems to be no evidence indicating that the dipolar ion form is any less likely than the neutral form, and since it clearly exists in this form in the solid, it appears most reasonable to assume that the dipolar ion form is the configuration in aqueous solution, until more definite evidence is forthcoming. It should be pointed out that it is, of course, quite possible that both forms are present in equilibrium.

If this is the case, then the equilibrium with the intermediate, X, postulated by Maron and Berens[7] in their discussion of the kinetics of the hydrolysis of sulfamic acid, is merely the dissociation equilibrium. It is interesting to note that the values of ΔH and ΔS given by King and King[1] for the dissociation equilibrium, when extrapolated to 90°, are of the correct sign required for Maron and Berens' explanation of their data. This is certainly not any argument in favor of the dipolar ion form over the neutral molecule form, but does show that there is no difficulty in this interpreta-

(1) E. J. King and G. W. King, THIS JOURNAL, **74**, 1212 (1952).
(2) P. Baumgarten, *Ber.*, **62B**, 820 (1929).
(3) F. A. Kanda and A. J. King, THIS JOURNAL, **73**, 2315 (1951).
(4) *Cf. C. A.*, **23**, 5159 (1929).
(5) G. E. K. Branch and M. Calvin, "Theory of Organic Chemistry," Prentice-Hall, New York, N. Y., 1941, p. 201.
(6) G. A. Jeffrey and H. P. Stadler, *J. Chem. Soc.*, 1467 (1951).
(7) S. H. Maron and A. R. Berens, THIS JOURNAL, **72**, 3571 (1950).

tion. It should be further pointed out that Baumgarten[2] suggested that the hydrolysis probably proceeds by way of the dipolar ion.

DEPARTMENT OF CHEMISTRY E. C. LINGAFELTER
UNIVERSITY OF WASHINGTON L. F. KELLS
SEATTLE 5, WASHINGTON H. V. TARTAR

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EVIDENCE OF NEW LINKAGES IN DEXTRANS

Sir:

We have been able to demonstrate that a significant fraction of the anhydroglucose units in a certain dextran apparently is not attacked by sodium metaperiodate at 25°. Previous investigations[1] of several dextrans showed that substantially all the units were attacked. Methylation studies[2] on dextrans so far investigated indicate that the principal glucosidic linkage is 1,6′, and that, in some cases, 1,4′-linkages are also present. Units at branch points carry linkages on both the 4- and 6-positions. Our results strongly suggest that this dextran contains units linked in the 3-positions, or both the 2- and 4-positions (branch points), or a combination of these possibilities.

This dextran, produced by *Leuconostoc mesenteroides* NRRL B-742, and purified by precipitation between 41 per cent. and 90 per cent. ethyl alcohol, consumed 1.43 moles of periodate and produced 0.64 mole of formic acid per anhydroglucose unit when oxidized at 25°[1] for 250 hours, at which time the consumption of oxidant and production of acid had ceased. Sixty-four per cent. of the glucopyranosyl units are therefore substituted only on the 6-position. Two moles of periodate are consumed by each unit so linked. The percentage of anhydroglucopyranose units consuming only one mole of periodate is then 15% [1.43 − (2 × 0.64)]. These are probably linked on the 4- and 6-positions. According to these calculations, the remaining 21 per cent. of the anhydroglucose units are not oxidized.

To confirm the presence of unoxidized units, a method developed by Smith[3] and his associates at the University of Minnesota has been applied. After removal of salts, the oxidized polymer was catalytically reduced and then hydrolyzed in 2 *N* sulfuric acid on the steam-bath. The only optically active products expected from a polyanhydroglucopyranose treated as above are D-glyceraldehyde, from 2- or 2- and 6-linked units, and D-glucose, from unoxidized units. The optical activity of the hydrolysate, if assumed to be due entirely to glucose, corresponded to 11.7% of unoxidized anhydroglucose units in the original dextran. Catalytic reduction of the neutralized hydrolysate yielded a solution having a small negative optical rotation in good agreement with that expected from the conversion of glucose to sorbitol. Sorbitol was isolated as the pyridine complex[4] and characterized as the hexaacetate, m.p. and mixed m.p., 98–99°; $[\alpha]^{25}D + 10.0°$ (*c*, 3.8; $CHCl_3$). The yield of the hexaacetate corresponded to 5.8% unoxidized anhydroglucopyranose in the original dextran.

The simplest explanation for the lack of oxidation by periodate is the presence of 1,3′-glucosidic linkages. Linkage in the 3-position, regardless of other linkages on the same anhydroglucopyranosyl unit, would prevent oxidation. Oxidation wuld be prevented also by the presence of units at branch points linked in both the 2- and 4-positions. However, the fact that the optical activity of the reduced hydrolysate indicated conversion of D-glucose to sorbitol, rather than of D-glyceraldehyde to glycerol, seems to rule out the presence of 1,2′-glucosidic linkages. Hence, if any 2-linked units are present, they probably occur only at branch points.

Dextran from *L. mesenteroides* NRRL B-742 has been found by Dr. Hellman at this Laboratory to consist of at least two discrete fractions.[5] Periodate analysis of the less soluble fraction, *i.e.*, that portion *precipitated* by 41% ethyl alcohol, does not indicate the presence of unoxidized anhydroglucose units. The fractions have been found by other workers here to differ also in specific rotation, viscosity, and infrared absorption.

Periodate oxidation data on dextrans produced by several other organisms have exhibited similar indications of unoxidized anhydroglucose units. In those cases where calculations indicate the presence of such units, unusual infrared absorption[6] is also found.

Methylation studies are in progress at this Laboratory to establish the positions involved in glycosidic linkage.

STARCH AND DEXTROSE DIVISION
NORTHERN REGIONAL RESEARCH LABORATORY[7]
PEORIA, ILLINOIS ROLLAND LOHMAR

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(1) Allene Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(2) M. Stacey and C. R. Ricketts, *Fortschr. Chem. Org. Naturstoffe*, **8**, 28 (1951).

(3) F. Smith, personal communication.

(4) H. H. Strain, THIS JOURNAL, **56**, 1756 (1934).

(5) N. N. Hellman, in "Report of Working Conference on Dextran," National Research Council, Subcommittee on Shock, and Northern Regional Research Laboratory, Peoria, Illinois, Oct. 29, 1951, p. 36.

(6) S. C. Burket and E. H. Melvin, *Science*, **115**, 516 (1952).

(7) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

STEREOSPECIFIC TOTAL SYNTHESIS OF CORTISONE

Sir:

We should like to report a stereospecific[1] total synthesis of 11-ketoprogesterone, dehydrocorticosterone and cortisone in both the natural and *dl* modifications. *dl*-4b-Methyl-7-ethylenedioxy-1, 2,-3, 4, 4aα, 4b, 5, 6, 7, 8, 10, 10aβ-dodecahydrophenanthrene-4β-ol-1-one[2] (I) with methyl iodide and potassium *t*-butoxide gave the 2-methyl derivative, m.p. 189–192°. *Anal.* Found: C, 70.58; H, 8.42. The latter was alkylated in turn with methallyl iodide to give 2β,4b-dimethyl-2-meth-

(1) "Stereospecific" is taken to mean that in each reaction producing a fixed asymmetric center, the ratio of isomer having the same configuration as the end product to all other isomers is greater than unity. In point of fact, each of such ratios in the present synthesis is 8:1 or greater.

(2) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, in press.