Calcd. for $C_{12}H_{18}BrNO_2S$: C, 46.45; H, 2.60. Found: C, 46.41; H, 2.70.) Reduction furnished 2-amino-2′-bromodiphenyl sulfide, m.p. 62–63°. (*Anal.* Calcd. for $C_{12}H_{10}BrNS$: C, 51.44; H, 3.60. Found: C, 51.00; H, 3.63. Hydrochloride, m.p. 132–134°. *Anal.* Calcd. for $C_{12}H_{11}BrClNS$: C, 45.51; H, 3.50. Found: C, 45.57; H, 3.79.) Benz-*o*-chloroanilide[6] and *o*-chlorophenylacetone[7] were made by standard methods. Acetoacet-*o*-chloroanilide was the product of Union Carbide Chemicals Co. Products were identified by mixed melting points with authentic samples or by comparison of melting points of the product and at least one derivative thereof with literature values. The yields reported are not considered to be optimum.

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RECEIVED MARCH 21, 1958

THE SITE OF CLEAVAGE OF *MYO*-INOSITOL BY PURIFIED ENZYMES OF RAT KIDNEY

Sir:

We have reported previously[1] the cleavage and conversion of inositol to glucuronic acid (racemic mixture) by rat kidney extracts. The enzyme preparation has now been purified, and the system that forms D-glucuronate has been obtained free of the one that forms the L-isomer. Resolution was obtained by treatment with calcium phosphate gel which adsorbs only the system that forms the L-isomer. The enzyme system forming the D-isomer was further purified 200-fold. Attempts to elute the adsorbed enzyme from the gel produced inactive preparations. Employing inositol-2-C^{14} [2] as the substrate and incubating separately with either the crude enzyme system or the purified enzyme capable of yielding only the D-isomer, we isolated 8 to 10 mg. of the respective radioactive glucuronic acids. They were converted to their lactones with m.p. of 176–178° for the racemic lactone[1] and 180° for the D-glucuronolactone having an $[\alpha]^{23}D$ +18.6° (*c* 1 in H_2O, *l* = 1 dm.). The radioactive glucuronolactones were diluted fivefold with the respective non-radioactive glucuronolactones and crystallized to a constant specific activity of approximately 1755 c.p.m. per micromole. The distribution of C^{14} within each of the glucuronates was obtained as follows: the uronic acids were reduced with $NaBH_4$ to gulonic acids which were converted to their lactones. The lactone derived from the racemic glucuronate was optically inactive and could not be crystallized. The lactone of the D-isomer had a m.p. of 184–185° and an $[\alpha]^{23}D$ +56° as expected for L-gulonolactone (*c* 1 in H_2O, *l* = 1 dm.). The gulonolactones were titrated with NaOH and were oxidized with HIO_4 to 1 mole of formaldehyde, 1 mole of glyoxylate and 3 moles of formate per mole of gulonolactone. The formaldehyde was isolated as the dimedon derivative, m.p. 191°, in 95% yield. The formic acid was distilled and isolated as the sodium salt in 100% yield. The glyoxylate was obtained in 85% yield and was characterized as described earlier[3] and by its 2,4-dinitrophenylhydrazone, m.p. 192°. Aliquots of these fractions were plated and counted in a gas flow counter. More than 98.5% of the C^{14} of the degraded gulonolactones were recovered in the glyoxylate fraction. The radioactive glyoxylates were further oxidized with HIO_4 to equimolar amounts of CO_2 and formate.[3] This formate contained all the C^{14} of the glyoxylate. These results demonstrate that the C-2 of inositol becomes C-5 of the racemic as well as of the D-glucuronate. Thus, inositol cleavage by the purified enzyme system occurs between C-1 and C-6 to form *exclusively* D-glucuronate. The formation of L-glucuronate may result from cleavage of inositol between C-3 and C-4, assuming that no racemization occurs of a possible intermediate between inositol and D-glucuronate. Further studies are needed to confirm this latter mechanism.

(1) F. C. Charalampous and C. yras, *J. Biol. Chem.*, **228**, 1 (1957).
(2) We are grateful to Dr. Laurens Anderson for a generous gift of inositol-2-C^{14}.
(3) F. C. Charalampous, *J. Biol. Chem.*, **225**, 585 (1957).

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IDENTIFICATION OF CYANOACETIC ACID AS A METABOLITE OF β-AMINOPROPIONITRILE (BAPN) AND OTHER NITRILES[1]

Sir:

The administration of β-aminopropionitrile (BAPN) to rats or rabbits has been shown to result in the formation of a metabolite which could be detected in the urine or blood serum by an orange-pink spot on paper chromatograms developed with diazotized sulfanilic acid.[2] Efforts to isolate the metabolite from ethyl acetate extracts of acidified urine led to the separation of a colorless, crystalline, nitrogenous solid which was very soluble in water, highly acidic, distillable *in vacuo*, and exhibited the characteristic chromatographic behavior.[2] Further examination of this solid showed that it was not pure, but the metabolite was obtained from it in substantially pure form by ion-exchange chromatography on Dowex-1 resin (acetate form) and has now been identified as *cyanoacetic acid*. The isolated substance melted at 67–68°, undepressed by admixture with an authentic sample, and corresponded closely with known cyanoacetic acid in its infrared spectrum and chromatographic behavior on paper. *Anal.* Calcd. for $C_3H_3O_2N$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.33; H, 3.68; N, 15.42.

Cyanoacetic acid was also detected in the urine of rats following administration of 3,3′-iminodipropionitrile,[3,4] ethylene cyanohydrin and valeronitrile. Administered cyanoacetic acid was ex-

(1) Supported in part by grants A538(C8) and A1498 from the National Institutes of Health, U. S. Public Health Service.
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(4) H. A. Hartmann and H. F. Stich, *Science*, **125**, 445 (1957).