chain aldehydes in biological oxidations or peroxidations.

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THE OPTICAL ISOMERS OF HYDROXYLYSINE AND ALLOHYDROXYLYSINE

Sir:

The structure α,ϵ-diamino-δ-hydroxycaproic acid first proposed for hydroxylysine by Van Slyke and co-workers[1] was shown to be correct by two groups of workers.[2] Several syntheses[3] of hydroxylysine have been reported all of which presumably lead to a mixture of the two possible diastereoisomers.[4]

The only optical data reported for this amino acid are those of Sheehan and Bolhofer[5] in which variation in observed values (specific rotation varied from -4.5 to $+14.9°$ for a 2% soln. in 1*N* hydrochloric acid) was attributed to racemization during the isolation procedure. It seemed desirable therefore to prepare the optical antipodes of both hydroxylysine and allohydroxylysine utilizing the general enzymatic asymmetric hydrolysis procedure developed in this Laboratory.[6]

Forty grams of hydroxylysine[3d] was converted to 47 g. (79%) of the ϵ-N-carbobenzoxy derivative by the method Neuberger and Sanger[7] used to prepare the corresponding lysine derivative. *Anal.*[8] Calcd. for $C_{14}H_{20}O_5N_2$: C, 56.8; H, 6.8; N, 9.5. Found: C, 56.8; H, 6.9; N, 9.5. Forty-four grams of this material was chloroacetylated by the Schotten–Bauman procedure. Fractional crystallization of the product from ethyl acetate yielded the diastereoisomeric α-chloroacetamino-ϵ-carbobenzoxy-amino-δ-caprolactones. The first of these (A) obtained in 29% yield (16 g.) was derived from hydroxylysine (see below) and had m.p 150–152°.[9] *Anal.* Calcd. for $C_{16}H_{21}O_6N_2Cl$: C, 54.2; H, 5.4; N, 7.9. Found: C, 54.3; H, 5.5; N, 7.8. The other diastereoisomer (B) obtained in 22% yield (12 g.) derived from allohydroxylysine had m.p. 143–145°; *Anal.* Found: C, 54.4; H, 5.6; N, 7.7. The mixed m.p. of A and B was 125–130°.

Seventeen and seven-tenths grams of lactone (A) was neutralized, *p*H 7.2, with lithium hydroxide and 0.75 g. of acylase I[10] added. Digestion[6] was continued with the addition of more acylase I until 90% hydrolysis of the L-enantiomorph was indicated. Isolation of the product in the usual way[6] gave 3.2 g. of ϵ-N-carbobenzoxyhydroxylysine, $[\alpha]^{25}D$ $+8.8°$, *c* 2 in 6*N* hydrochloric acid. *Anal.* Found: C, 56.3; H, 6.8; N, 9.6. Hydrogenolysis of 2.5 g. of this material in the presence of hydrochloric acid and palladium black yielded 1.12 g. of L-hydroxylysine monohydrochloride, $[\alpha]^{25}D$ $+14.5°$ *c* 2 in 6*N* hydrochloric acid or $+17.8°$ for the free base.[11] *Anal.* Calcd. for $C_6H_{15}O_3NCl$: C, 36.2; H, 7.5; N, 14.1. Found: C, 36.6; H, 7.5; N, 13.7.

The D-enantiomorph was obtained by extraction of the acidified mother liquors from the isolation of the carbobenzoxy L-derivative, followed by hydrogenolysis of the carbobenzoxy group, and acid hydrolysis to remove the chloroacetyl group. The 3.2 g. of D-hydroxylysine obtained was shown by treatment of a small sample with lysine decarboxylase[12,13] to contain about 10% of the L-enantiomorph. This same decarboxylase was used to remove the L-isomer from the main fraction of the D compound thus yielding 2.8 g. of D-hydroxylysine monohydrochloride, $[\alpha]^{25}D$ -14.5, *c* 2 in 6*N* hydrochloric acid or $-17.8°$ for the free base. *Anal.* Found: C, 36.1; H, 7.8; N, 13.8.

Similar treatment of 16.5 g. of lactone (B) yielded 1.5 g. of ϵ-N-carbobenzoxyallohydroxylysine $[\alpha]^{25}D$ $+19.8$, *c* 2% in 6*N* hydrochloric acid. *Anal.* Found: C, 56.8; H, 7.8; N, 9.6. From 1.3 g. of this material there was obtained 0.45 g. of L-allohydroxylysine monohydrochloride, $[\alpha]^{25}D$ $+25.8°$, *c* 2 in 6*N* hydrochloric acid or $+31.4°$ for the free base. *Anal.* Found: C, 36.2; H, 7.7; N, 13.9. From the mother liquors 2.6 g. of the crude D-isomer (about 15% L) was obtained which upon purification with lysine decarboxylase gave 1.5 g. of pure D-allohydroxylysine monohydrochloride $[\alpha]^{25}D$ -26.3, *c* 2 in 6*N* hydrochloric acid or $-32.1°$ for the free base. *Anal.* Found: C, 36.1; H, 7.8; N, 14.0.

Both of the D-isomers were treated with benzoyl chloride using the procedure of Weisiger.[3b] From D-hydroxylysine there was isolated a dibenzoyl derivative, m.p. 168–170°, $[\alpha]^{25}D$ $-4.0°$, *c* 1% in ethanol. *Anal.* Calcd. for $C_{20}H_{22}O_5N_2$: C, 64.8; H, 5.9; N, 7.6. Found: C, 64.6; H, 6.1; N, 7.2. The melting point is in good agreement with those reported for this derivative prepared from hydroxylysine isolated from proteins (172°,[3b] 167–169°,[2b] 171–172°[14]) and the rotation agrees with that reported by Weisiger[3b] for N,N^1-dibenzoyl-D-hydroxylysine. The only crystalline product that could be isolated from the benzoylation of D-allohydroxylysine was one analyzing correctly for the corresponding lactone, m.p. 196–198°. *Anal.* Calcd. for $C_{20}H_{20}O_4N_2$: C, 68.2; H, 5.7; N, 8.0. Found: C, 68.6; H, 6.0; N, 7.6. On the basis of these facts provisional assignment

(1) D. D. Van Slyke, A. Hiller, D. A. MacFayden, A. B. Hastings, and F. W. Klemperer, *J. Biol. Chem.*, **133**, 287 (1940); F. W. Klemperer, A. B. Hastings and D. D. Van Slyke, *ibid.*, **143**, 433 (1942).

(2) (a) J. C. Sheehan and W. A. Bolhofer, THIS JOURNAL, **72**, 2769 (1950); (b) S. Bergstrom and S. Lindstedt, *Arch. Biochem.*, **26**, 323 (1950).

(3) (a) J. C. Sheehan and W. A. Bolhofer, THIS JOURNAL, **72**, 2472 (1950); (b) J. R. Weisiger, *J. Biol. Chem.*, **186**, 591 (1950); (c) O. Touster, THIS JOURNAL, **73**, 491 (1951); (d) G. Van Zyl, E. E. Van Tamelen and G. D. Zuidema, *ibid.*, **73**, 1765 (1951).

(4) Weisiger (ref. 3b) isolated only one diastereoisomer by a fractionation of his reaction product through the picrate though both diastereoisomers were presumably formed in the synthesis.

(5) J. C. Sheehan and W. A. Bolhofer, THIS JOURNAL, **72**, 2466 (1950).

(6) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, *J. Biol. Chem.*, **204**, 307 (1953), and preceding papers.

(7) A. Neuberger and F. Sanger, *Biochem. J.*, **37**, 515 (1943).

(8) Analyses by R. J. Kogel and staff of this Laboratory.

(9) All m.p.'s in capillary tubes and are corrected.

(10) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952).

(11) The specific rotation was the same in 1 *N* hydrochloric acid.[5]

(12) (a) E. F. Gale and H. M. R. Epps, *Biochem. J.*, **38**, 232 (1944); (b) S. Lindstedt, *Acta Chem. Scand.*, **5**, 486 (1951).

(13) The author is indebted to Dr. Alton Meister for generous amounts of lysine decarboxylase.

(14) L. K. Ramachandran and P. S. Sarma, *J. Sci. Ind. Research*, **12**, 4 (1953).

of the names, hydroxylysine and allohydroxylysine, to the pure isomers has been made.

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(15) U. S. Public Health Service, Department of Health, Education and Welfare.

LOW MOLECULAR WEIGHT DEXTRAN AS A MODIFIER OF DEXTRAN SYNTHESIS[1]

Sir:

Dextran synthesis by *Leuconostoc mesenteroides* is brought about by a special enzyme, dextransucrase, that apparently causes the direct transfer of α-D-glucopyranosyl radicals from many sucrose molecules to a few acceptor molecules which become growing dextran chains.[2] Following the report of Koepsell, *et al.*,[3] that certain sugars can serve as "alternate" acceptors for dextransucrase, we[4] and independently Tsuchiya, Hellman and Koepsell[5] have found that low molecular weight dextrans also serve as acceptors. An especially significant point revealed by our experiments is that small-sized dextrans are intrinsically much more potent modifiers of dextran synthesis than such sugars as maltose or glucose.

To compare the modifying action of dextrans and sugars, dextransucrase from *L. mesenteroides* strain B[2] was incubated at 25° and *p*H 5.0 with sucrose (50 mg./ml. final concentration) and the substance to be tested as a supplement. Individual controls were prepared, comprising enzyme and sucrose incubated together, with the supplementary substance added after incubation. The final mixtures were analyzed for dextran and oligosaccharide contents (Fig. 1).

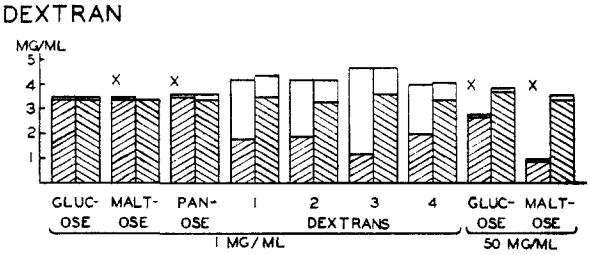

Fig. 1.—Low molecular weight dextrans *vs.* sugars as modifiers of dextran synthesis. Analyses of incubated enzyme–sucrose–supplement mixtures shown at the left of analyses of control enzyme–sucrose mixtures with the supplement added after incubation. Hatched area show dextran precipitated at 25° by 40% (v./v.) methanol; open areas show dextran precipitated between 40 and 65% methanol; × indicates oligosaccharide(s), other than sucrose or supplement, detected by chromatography.[3] Dextrans 1 and 2 were clinical products, kindly supplied by Commercial Solvents Corp., Terre Haute, Ind., and Dextran Corp., Yonkers, N. Y.; dextrans 3 and 4 were fractions of mol. wt. *ca.* 20,000 and 18,000 kindly supplied by Dr. F. R. Senti and Dr. B. Ingelman.

At 1 mg./ml., glucose as a supplement had no detectable effect on the dextran polymerization, while maltose and crystalline panose (kindly supplied by Dr. S. C. Pan) induced formation only of traces of oligosaccharides. "Normal" dextran, precipitable by 40% methanol, was synthesized to the same extent in the presence as in the absence of the three sugars. In contrast, each of four different low molecular weight dextrans (actually fractions of partly hydrolyzed dextrans) profoundly affected the synthesis. Mixtures incubated with these supplements contained smaller amounts of dextran precipitated by 40% methanol than the controls, and had appreciable contents of presumably lower molecular weight dextran, precipitating between 40 and 65% methanol. In the control mixtures, most of the 40 to 65% fraction represents added dextran supplement.

The modifying effect of the 1 mg./ml. dextran supplements was greater than that of 50 mg./ml. glucose and, in at least one instance, approached that caused by 50 mg./ml. maltose. Taking molecular concentration into account, it is evident that the acceptor capacity of an individual small dextran molecule is exceedingly high and of a different order of magnitude than the sugars tested. Attention is directed to this capacity as one factor that may enable useful modifications of dextran synthesis to be made.

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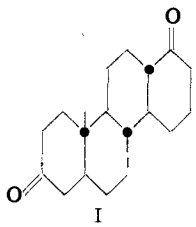
18-NOR-D-HOMOANDROSTANE-3,17a-DIONE

Sir:

The (±) diketone I, m.p. 149–150.5° cor. (Found: C, 79.0; H, 9.91) has been prepared by chromic acid oxidation of 18-nor-D-homoepiandrosterone which, as already reported,[1] is readily made in five operations from 2,5-dimethoxynaphthalene.



I

Androgenic assays in rats performed under the direction of Drs. R. K. Meyer and Elva G. Shipley of the Department of Zoölogy show this racemic compound to be at least one-tenth as active as testosterone. Since androstane-3,17-dione itself

(1) Supported by a grant from the Corn Industries Research Foundation.

(2) E. J. Hehre, *Proc. Soc. Exp. Biol. Med.*, **54**, 240 (1943); *J. Biol. Chem.*, **163**, 221 (1946); "Advances in Enzymology," Interscience Publishers, Inc., New York, N. Y., **11**, 297 (1951).

(3) H. J. Koepsell, H. M. Tsuchiya, N. N. Hellman, A. Kazenko, L. A. Hoffman, E. S. Sharpe and R. W. Jackson, *Bact. Proc.*, 24 (1952); *J. Biol. Chem.*, **200**, 793 (1953).

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