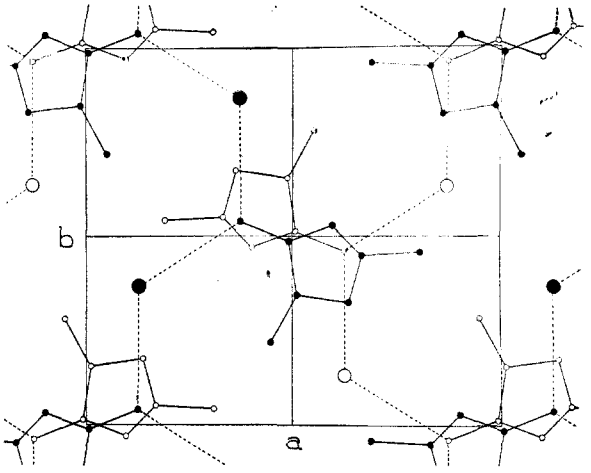

Fig. 1.—*Projected arrangement of molecules of 1,3-dimethyl-5-iminotetrazole hydrochloride in one unit cell of crystal.* Filled circles represent atoms in the plane $Z = {}^3/_4$; open circles represent atoms in the plane $Z = {}^1/_4$. The large circles are chloride ions. The broken lines are hydrogen bonds between chloride ions and imino nitrogens.

ber of resonance possibilities. The strong basic character of the compound as well as the planar structure of the molecule are consistent with these ideas. Support for this resonance hybrid structure is found in the fact that the hydrochloride shows an absorption in the ultraviolet ($\lambda_{max.}^{H_2O} = 254$ mμ, $\epsilon = 2600$) whereas normal tetrazoles show only end absorption[5] when the substituents possess no conjugation.

The crystal structure of the hydrochloride salt is being refined by three-dimensional methods to obtain more accurate bond lengths. However, the present state of the Fourier synthesis of the structure is such as to rule out unequivocably the possibility of a bridged ring compound. The details of the structure determination will be reported elsewhere.

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RECEIVED AUGUST 31, 1953

THE IDENTIFICATION OF KCF: REQUIREMENT OF LONG-CHAIN ALDEHYDES FOR BACTERIAL EXTRACT LUMINESCENCE[1]

Sir:

It has been reported earlier that diphosphopyridine nucleotide or its reduced analog will support the luminescence of cell- and particle-free extracts of the luminous bacterium, *Achromobacter fischeri*.[2] More recently we have demonstrated the requirement for two additional factors for optimal luminescence.[3] One of these components is flavin mononucleotide, the other, a factor obtainable from kidney cortex powders, we have called KCF (kidney cortex factor). McElroy and coworkers (personal communication) have also demonstrated a requirement for riboflavin phosphate and for another unidentified factor which they have considered to be bacterial luciferin by analogy with firefly luciferin,[4] since it was apparently destroyed during luminescence.

We can now report the identification of KCF as plasmal,[5] or specifically, palmitaldehyde. This substance, purified through various partition and precipitation procedures was capable of producing 5- to 10-fold increase (maximum increase = 100×) in the luminescence of *A. fischeri* extracts at a concentration of 5×10^{-7} g./ml. and an authentic sample of this C_{16} aldehyde (prepared by the Rosenmund reduction of the acid chloride[6]) was found to replace KCF quantitatively. Other long chain fatty aldehydes ($C_{7,8,9,11,18}$) are also active, but shorter homologs are inactive ($C_{2,4}$).

During the isolation procedures a strong fuchsin-aldehyde test was found to parallel biological activity while the general physical and chemical properties suggested an aliphatic lipid. The 2,4-dinitrophenylhydrazone of KCF was prepared and identified by elementary analysis, molecular weight, and mixed melting points.[7] That the biological activity is dependent on a free aldehyde group is shown by the disappearance of an aldehyde test under conditions where enzymatic activity was also destroyed.

It remains to be demonstrated that long-chain fatty aldehydes are responsible for the activity associated with the heat-precipitable fractions obtained from *A. fischeri*,[3] but it would seem unlikely that KCF is a luciferin analogous to firefly luciferin. Rather, the compound in *A. fischeri* most closely resembling this fluorescent firefly component would seem to be riboflavin phosphate, a compound known to chemiluminesce in the presence of peroxide,[8] and to be involved in the luminescence of the earthworm, *Eisenia submontana*.[9] The striking similarity in certain physical properties, *e.g.*, fluorescence and infrared absorption spectrum, between riboflavin and firefly and firefly luciferin would tend to substantiate this hypothesis.[10]

While the mechanism of action of long-chain fatty aldehydes on luminescence remains to be elucidated, the participation of the long-known Feulgen-positive components,[5] in an obligatory respiratory reaction such as bioluminescence raises the question of the general functions of long-

(1) Work performed under contract No. W-4705-eng-26 for the Atomic Energy Commission.

(2) B. L. Strehler, THIS JOURNAL, **75**, 1264 (1953).

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(4) B. L. Strehler and W. D. McElroy, *J. Cellular Comp. Physiol.*, **34**, 457 (1949).

(5) R. Feulgen, K. Imhäuser and M. Behrens, *Z. physiol. Chem.*, **180**, 161 (1929).

(6) K. W. Rosenmund, *Ber.*, **51**, 585 (1918).

(7) Analytical data on dinitrophenylhydrazone of KCF: m.p. KCF, 104.5–105.6°; palmitaldehyde, 105.2–106°; mixed m.p. 105–106°; mol. wt. calcd. from extinction, 431; palmitaldehyde, 420; analysis, calcd. for $C_{22}H_{36}N_4O_4$: C, 62.86; H, 8.57; N, 13.33; found: C, 63.11; H, 8.86; N, 13.57.

(8) B. L. Strehler and C. S. Shoup, *Arch. Biochem. and Biophys.*, in press.

(9) J. Komarek and K. Wenig, Věstnik čsl. Spolec. Nauk. (article 12), 1–12 (1938).

(10) B. L. Strehler and W. D. McElroy, unpublished.

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

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