mineral metabolism and suggested that it may be a desoxycorticosterone with one extra hydroxyl group in close proximity to the side chain. Since Professor Reichstein and his collaborators have just announced the isolation of crystalline electrocortin, we are presenting our findings so that the physical properties of I can be compared with those of acetylated electrocortin.

3β-Formyloxy-16α-benzyloxy-Δ⁵-pregnen-20-one[2] (m.p. 130.5–131°, $[\alpha]_D$ −44°, chf. *Anal.* Calcd. for $C_{29}H_{38}O_4$: C, 77.30; H, 8.50. Found: C, 77.46; H, 8.63) in 95% ethanol on hydrogenation with palladium on charcoal and acetylation gave 3β-formyloxy-16α-acetoxy-Δ¹-pregnen-20-one (m.p. 195–197.5 ± 2°, $[\alpha]_D$ −45°, chf. *Anal.* Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.77; H, 8.54; infrared (all measurements in carbon disulfide) maxima at ~ 8.48, 5.79 (formate), 8.03, 5.74 (acetate), 5.84 (ketone) (the carbonyl peaks were not fully resolved), 12.45, 12.22 μ (Δ⁵). Treatment with lead tetracetate in acetic acid containing acetic anhydride introduced one acetoxy group into the 21-position as deduced from the composition (*Anal.* Calcd. for $C_{26}H_{36}O_7$: C, 67.80; H, 7.88. Found: C, 67.98; H, 7.98), the change in molecular rotation (+75°) and the infrared spectrum (two acetate peaks[4] at 8.04 and 8.12 μ, a very intense unresolved peak at 5.78 μ (16-acetate, formate and 20-ketone[4]) with shoulder at 5.71 μ (21-acetate)). Moreover, 3β-formyloxy, 16α,21-diacetoxy-Δ⁵-pregnen-20-one (m.p. 180.5–182.5°, $[\alpha]_D$ −23°, chf.) reduced 2,3,5-triphenyl-2H-tetrazolium chloride. The newly introduced acetoxy group was hydrolyzed by dilute potassium bicarbonate at 22° at a rate comparable to that of desoxycorticosterone acetate. Brief exposure, however, gave as the main product 16α,21-diacetoxy-Δ⁵-pregnen-3β-ol-20-one (m.p. 158–160°; infrared, no formate peak near 8.48 μ, maxima at 8.05, 8.12, 5.76, 5.71 (shoulder), 2.78 μ (unassociated hydroxy). Treatment with bromine, chromium trioxide and chromous chloride gave I (m.p. 150.5–153°, $[\alpha]_D$ +110° alc. (*c*, 0.3). *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.65; H, 7.93. Absorption maxima: 8.12, 8.05, 5.95 (Δ⁴-3-ketone), 5.70 μ (shoulder) and 240 mμ (ε = 16400 alc.).

Enzymatic hydrolysis with cholinesterase from red cells yielded a product which migrated in a paper chromatogram (propylene glycol–toluene) at a rate very close to that of a highly potent sodium retaining factor from canine adrenal venous blood.[5] Further characterization of the hydrolyzed product awaits the preparation of additional material.[6]

DEPARTMENT OF MEDICINE H. HIRSCHMANN
DEPARTMENT OF PHYSIOLOGY FRIEDA B. HIRSCHMANN
WESTERN RESERVE UNIVERSITY GORDON L. FARREL
CLEVELAND 6, OHIO

RECEIVED SEPTEMBER 3, 1953

(4) For spectra of 21-acetoxy-20-ketones see R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, THIS JOURNAL, **70**, 2024 (1948).

(5) G. L. Farrell and J. B. Richards, *Proc. Soc. Exp. Biol. and Med.*, **83**, 628 (1953).

(6) Addendum September 19, 1953.—The hydrolytic product has been assayed by Dr. John Luetscher, Jr., Stanford University, and Dr. Paul Royce. The tests have failed to demonstrate the high salt retaining activity of electrocortin.

1,3-DIMETHYL-5-IMINOTETRAZOLE, A NEW CYCLIC "MESO-IONIC" COMPOUND

Sir:

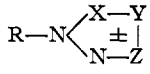
2-Methyl-5-aminotetrazole (m.p. 104.5–105.5°; C, 24.60; H, 5.04; N, 71.33) is obtained in 25–35% yield, together with 35–50% of the 1-isomer,[1] when an aqueous solution of sodium 5-aminotetrazole (1 mole) is heated with dimethyl sulfate (0.5 mole). When the 2-isomer is alkylated with methyl benzenesulfonate according to the procedure of Herbst, Roberts and Harvill,[2] there is recovered in 85% yield a basic, hygroscopic dimethyl derivative which melts at 88–90° after several recystallizations, first from benzene and finally from methylene chloride–petroleum ether (C, 32.07; H, 6.00; N, 61.92). This compound is different than *2-methyl-5-methylaminotetrazole* (m.p. 48–49°; N, 62.04). The *hydrochloride* (C, 24.16; H, 5.24; N, 47.08; Cl, 23.90) melts at 203–205° after recrystallization from 90% isopropyl alcohol.

The structure of this compound was established as *1,3-dimethyl-5-iminotetrazole*, instead of the anticipated 1,2-dimethyl derivative, by analysis of the X-ray data obtained on crystals of the hydrochloride and hydrobromide. These salts are orthorhombic and have the following cell constants:

	a_0, Å.	b_0, Å.	c_0, Å.
HCl salt	10.90	9.60	6.58
HBr salt	11.21	9.82	6.71

There are four molecules per unit cell, and the space group is Pbnm. The symmetry of the rotation photograph obtained about the *c*-axis shows that the molecules lie in mirror planes and hence are completely planar. The layer nature of the crystal is also shown by the great intensity of all orders of (001). Since the two crystals are isomorphous, it is possible to obtain the structure without any prior assumptions, by first using crystal data from the hydrobromide, and then applying the results to the data from the hydrochloride. Figure 1 shows the structure of the crystal as obtained by this procedure. The imino nitrogen was identified primarily by its being at distances of 3.15 Å. and 3.18 Å. from two chloride ions. This is the usual N-H · · · Cl bond length that has been found in a number of hydrochloride salts.[3]

This tetrazole derivative appears to belong to a class of compounds which have been called "cyclic meso-ionic" compounds,[4] and which have been given the general formula



The syndones were the first group of this type to be well characterized.[4] In our case, X is N, Y–Z is NRC(:NH), and R is CH_3; this is one of the predicted types.[4]

A large number of possible resonance structures can be written for this substance. In addition, the existence of the $=NH_2^+$ group increases the num-

(1) R. Stolle, *et al.*, *J. prakt. Chem.*, **134**, 282 (1932).

(2) R. Herbst, C. Roberts and E. Harvill, *J. Org. Chem.*, **16**, 139 (1951).

(3) J. Donohue, *J. Phys. Chem.*, **56**, 502 (1952).

(4) W. Baker, W. D. Ollis and V. D. Poole, *J. Chem. Soc.*, 307 (1949); R. A. W. Hill and L. E. Sutton, *ibid.*, 746 (1949).

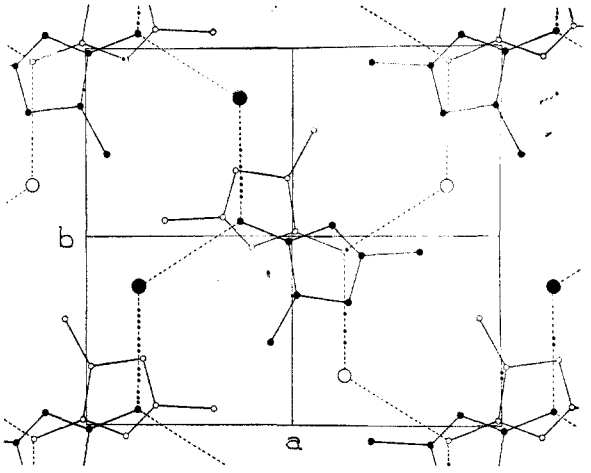


Fig. 1.—*Projected arrangement of molecules of 1,3-dimethyl-5-iminotetrazole hydrochloride in one unit cell of crystal.* Filled circles represent atoms in the plane $Z = {}^3/_4$; open circles represent atoms in the plane $Z = {}^1/_4$. The large circles are chloride ions. The broken lines are hydrogen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

(5) B. Elpern and F. C. Nachod, THIS JOURNAL, **72**, 3379 (1950); B. Elpern, *ibid.*, **75**, 661 (1953).

JOHN H. BRYDEN
RONALD A. HENRY
CHEMISTRY DIVISION WILLIAM G. FINNEGAN
U. S. NAVAL ORDNANCE TEST STATION ROBERT H. BOSCHAN
CHINA LAKE, CALIF. WM. S. MCEWAN
ROBERT W. VAN DOLAH

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).

(3) B. L. Strehler and M. J. Cormier, *Arch. Biochem. and Biophys.*, in press.

(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.