# COMMUNICATIONS TO THE EDITOR

## *p*-HYDROXYPHENYLPYRUVIC ACID FUNCTION IN *NEUROSPOSA CRASSA*[1]

*Sir:*

Investigation of a mutant strain of *Neurospora crassa* (T-145)[2] requiring tyrosine for growth ($2 \times 10^{-4}$ molar for maximum growth equal to wild type) revealed that the addition of amino acids at relatively low concentrations resulted in unusual growth inhibitions in the presence of tyrosine. These amino acids proved to be competitive with tyrosine over a wide concentration range.

Studies of individual L-isomers of the inhibitory amino acids revealed the inhibition indices to fall in the range of 5–250. The D-isomers inhibited in a competitive manner also, but had a higher index. While phenylalanine was inhibitory (inhibition index = 8) it was found that the related compounds, phenylpyruvic acid and phenyllactic acid, showed no inhibition. Of 23 L-isomers of amino acids tested for competitive inhibition with L-tyrosine, eighteen showed complete inhibition of growth within the relative substrate-inhibitor concentrations indicated above while five caused no inhibition at any concentration. The inhibitory amino acids are: alanine, $\alpha$-aminobutyric acid, aspartic acid, citrulline, cysteine, glycine, glutamic acid, histidine, isoleucine, leucine, methionine, norleucine, norvaline, phenylalanine, serine, threonine, tryptophan and valine; non-inhibitory amino acids are arginine, hydroxyproline, lysine, ornithine and proline.

Examination of the two groups of amino acids suggested at once that a major system such as transamination might be involved. This led to the testing of the keto analog of tyrosine (*p*-hydroxyphenylpyruvic acid).[3] These studies revealed that *p*-hydroxyphenylpyruvic acid completely satisfies the growth requirements of the mutant. Even more significant is the fact that it completely reverses the inhibition of the above amino acids in the presence of tyrosine. Relief of the inhibition occurred over a wide range of concentration (up to 100 times the concentration required for complete inhibition) of the inhibiting amino acid. The $\alpha$-keto analog of tyrosine shows activity in both cases mentioned above within the same concentration range in which tyrosine is active alone.

A suggested hypothesis that would explain all the data is one in which the inhibitory amino acids block the conversion of tyrosine to its keto analog which is normally utilized by the organism for some essential function. The exact nature of the genetic block is not clear but the evidence is strong in favor of the conclusion that the keto acid serves some essential function other than the formation of tyrosine.

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RECEIVED AUGUST 24, 1953

(1) This work was aided by a grant from the Office of Naval Research, United States Navy Department, administered by the University of Texas under contract Nonr 859(00).

(2) Obtained by A. Gib DeBusk, J. M. Weaver and R, A. McRorie in conjunction with the Genetics Group, University of Texas, by means of ultraviolet irradiation.

(3) We are indebted to Dr. Alton Meister of the Department of Health, Education and Welfare, Public Health Service, National Institute of Health, Bethesda 14, Maryland, for a sample of this compound for preliminary studies with this mutant.

## CONFIGURATION OF DIHYDROSPHINGOSINE

*Sir:*

In a recent communication[1] we presented evidence for the *erythro* configuration of the natural dihydrosphingosine. We have now completed the resolution of the synthetic *erythro*-1,3-dihydroxy-2-aminoöctadecane, and one of the optical isomers is identical with the natural base, as shown in the table:

| | | Triacetyl m.p. °C. | Specific rotation |
|---|---|---|---|
| Natural dihydrosphingosine | | 100–102 | $[\alpha]^{30}D$ +18° (chf.) |
| Synthetic enantiomorph | *erythro* | 98–100 | $[\alpha]^{22}D$ +19.2 (chf.) |
| | *threo* | 46 | ..... |

On the basis of these data and in conjunction with a previous report on the configuration of the amino carbon of dihydrosphingosine[2] it is now conclusively established that the natural dihydrosphingosine is *erythro*-D-1,3-dihydroxy-2-aminoöctadecane.

**Experimental.**—A warm solution of *erythro*-1,3-dihydroxy-2-aminoöctadecane (1.5 g.) in 50 cc. of ethanol was added to a warm solution of 740 mg. of finely powdered L-glutamic acid in 95 cc. of 50% ethanol. The slightly turbid solution was evaporated *in vacuo* to dryness. Vigorous foaming at the end of the distillation was overcome by adding two or three 50-cc. portions of ethanol. The dry salt was dissolved in 250 cc. of 90% ethanol (90 cc. ethanol–10 cc. distilled water) and left overnight at 20–22°; 750 mg. of a crystalline salt was filtered off. From the mother liquor 300 mg. more was separated after 24 hours. The combined solids were recrystallized once from 150 cc. and twice from 125 cc. portions of 96% ethanol and finally from 100 cc. of 90% ethanol. The pure salt melted incompletely at 136–140° and decomposed at 165–170°. The salt was decomposed with sodium carbonate and extracted with ether. The free base was converted into the triacetyl derivative which after two crystallizations from ethanol melted at 98–100°; $[\alpha]^{22}D$ −19.35 (0.066 g. in 10 cc. of chloroform).

The first two mother liquors from the above salt were concentrated. The residual salt was dis-

(1) H. E. Carter, D. Shapiro, J. B. Harrison, THIS JOURNAL, **75**, 1007 (1953).

(2) H. E. Carter and C. G. Humiston, *J. Biol. Chem.*, **191**, 727 (1950).

solved in water and heated with sodium carbonate. The liberated base was extracted with ether. The ethereal solution was washed thoroughly with water until neutral and evaporated. The base was converted into the D-glutamic acid salt and purified as described above. The purified base gave a triacetyl derivative melting at 98–100° ($[\alpha]^{22}D$ +19.2 (0.1 g. in 10 cc. of chloroform)).

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RECEIVED SEPTEMBER 21, 1953

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## THE PREPARATION OF D-HOMOPROGESTERONE AND D-HOMO-11-DEOXYCORTICOSTERONE ACETATE

*Sir:*

The sustained interest in the cortical hormones made it desirable to determine the effect of a six-membered D ring on cortical-hormonal activity. D-Homoprogesterone and D-homo-11-deoxycorticosterone acetate were prepared as the first part of this program.

Ethynylation of 3β-hydroxy-D-homoandrost-5-en-17a-one[1] (I) produced D-homo-17aα-pregn-5-en-20-yne-3β,17aβ-diol (II), m.p. 262–264°; $[\alpha]^{23}D$ −108° (0.5% in $CHCl_3$); (*Anal.* Calcd. for $C_{22}H_{32}O_2$: C, 80.4; H, 9.8. Found: C, 80.2; H, 9.8); and, in low yield, D-homopregn-5-en-20-yne-3β,17aα-diol (III), m.p. 220–222°; $[\alpha]^{23}D$ −76° (1% in $CHCl_3$); (*Anal.* Found: C, 80.2; H, 10.0.) Treatment of either II or III with formic acid[2] gave, after hydrolysis, 3β-hydroxy-D-homopregna-5,17(17a)-dien-20-one (IV), m.p. 233–235°; $[\alpha]^{23}D$ +35° (0.5% in $CHCl_3$); $\lambda_{max.}$ 233 mμ, ε 8,930; (*Anal.* Calcd. for $C_{22}H_{32}O_2$: C, 80.4; H, 9.8. Found: C, 80.7; H, 9.9.) plus an unidentified compound, $C_{22}H_{30}O$, m.p. 171–172°. Hydrogenation of IV yielded 3β-hydroxy-D-homopregn-5-en-20-one (V), m.p. 205–206°, $[\alpha]^{23}D$ −25° (1% in $CHCl_3$); (*Anal.* Calcd. for $C_{22}H_{34}O_2$: C, 80.0; H, 10.4. Found: C, 79.8; H, 10.3) which on Oppenauer oxidation gave the desired D-homoprogesterone, m.p. 158–160°; $[\alpha]^{23}D$ +167° (1% in $CHCl_3$); $\lambda_{max.}$ 242 mμ, ε 16,600; (*Anal.* Calcd. for $C_{22}H_{32}O_2$: C, 80.4; H, 9.8. Found: C, 80.5; H, 9.6.) Since attempts to isomerize D-homoprogesterone, by heating in acidic and in basic solution, failed, the configuration of the side chain at 17a is probably β.

Perfusion of D-homoprogesterone through surviving adrenal glands yielded neither D-homocorticosterone nor 17aα-hydroxy-D-homocorticosterone.

D-Homo-11-deoxycorticosterone acetate was prepared from V by use of the method devised by H. Ruschig.[3] Compound V was condensed with dimethyl oxalate using sodium methoxide in benzene. The sodium enolate so obtained was iodinated in methanol at −15°, then cleaved to 3β-hydroxy-21-iodo-D-homopregn-5-en-20-one (VI) with sodium methoxide at room temperature. The crude iodo compound (VI) was converted, by means of potassium acetate in acetone, to 3β,21-dihydroxy-D-homopregn-5-en-20-one 21-acetate (VII), m.p. 188–190°; (*Anal.* Calcd. for $C_{24}H_{36}O_4$: C, 74.2; H, 9.3. Found: C, 74.3; H, 9.6.) Oppenauer oxidation of VII yielded D-homo-11-deoxycorticosterone acetate (VIII), m.p. 152–154°; $[\alpha]^{23}D$ +150° (0.45% in $CHCl_3$); $\lambda_{max.}$ 241 mμ, ε 16,200; (*Anal.* Calcd. for $C_{24}H_{34}O_4$: C, 74.6; H, 8.9. Found: C, 74.9; H, 8.9). Compound VIII showed no appreciable ability to prevent sodium excretion in adrenalectomized rats,[4] but it did possess approximately 10% of the activity of 11-deoxycorticosterone acetate in the maintenance of life in adrenalectomized rats on a sodium deficient diet.[5]

(1) H. Heusser, P. Th. Herzig, A. Fürst and Pl. A. Plattner, *Helv. Chim. Acta*, **33**, 1093 (1950).

(2) J. D. Chanley, THIS JOURNAL, **70**, 244 (1948).

(3) H. Ruschig, U. S. pat. 2,609,379, Sept. 2, 1952.

(4) C. M. Kagawa, E. G. Shipley and R. K. Meyer, *Proc. Soc. Exptl. Biol. and Med.*, **80**, 281 (1952).

(5) A. Grollman, *Endocrinology*, **29**, 855 (1941). We are indebted to F. J. Saunders, C. G. Van Arman and C. M. Kagawa of our Laboratories for the determination of biological activities.

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RECEIVED SEPTEMBER 24, 1953

## MAGNETIC CATALYSIS OF A DECARBOXYLATION REACTION[1]

*Sir:*

There is now a considerable body of experimental evidence[2] that the rate of decarboxylation of $C^{13}$ substituted carboxylic acids is appreciably higher that would be expected from the rate of decarboxylation of the $C^{12}$- and $C^{14}$-compounds on the basis of change of isotopic mass alone.

A possible cause of this apparent anomaly could lie in the nonzero nuclear spin and magnetic moment of $C^{13}$. Both $C^{12}$ and $C^{14}$ have zero values for these properties. A paramagnetic rare earth ion such as dysprosium at a distance of a few angströms from the C–C bond could cause an inhomogeneous magnetic field comparable to that caused by a $C^{13}$ nucleus at one end of the bond.

We have now found such an acceleration of the rate of decarboxylation of (natural) phenylmalonic acid in aqueous solution at 45° in the presence of 0.5 *N* dysprosium ion.

The kinetics of the decarboxylation of phenylmalonic acid have been explored.[3] The conditions selected for the present experiments, *p*H 0.4–0.8, yield a first order reaction of un-ionized phenylmalonic acid to phenylacetic acid with a rate almost independent of *p*H. Experiments were carried out with phenylmalonic acid alone and in the presence of 0.5 *N* $La^{3+}$, $Y^{3+}$ and $Dy^{3+}$ as rare earth chlorides. The initial *p*H of the reaction mixtures was equalized by addition of standard hydrochloric acid. A dozen aliquots were withdrawn at intervals during the first 50% of reaction,

(1) This work was assisted by the American Petroleum Institute through Research Project 50. The dysprosium was kindly made available to us by Dr. F. H. Spedding.

(2) P. E. Yankwich and E. C. Stivers, *J. Chem. Phys.*, **21**, 61 (1953).

(3) E. Gelles, accepted for publication, THIS JOURNAL.