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 (2×10^{-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, α-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 α-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.

BIOCHEMICAL GENETICS LABORATORIES
DEPARTMENT OF ZOOLOGY — A. GIB DEBUSK
UNIVERSITY OF TEXAS — ROBERT P. WAGNER
AUSTIN, TEXAS

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)).

DIVISION OF BIOCHEMISTRY HERBERT E. CARTER
NOYES LABORATORY OF CHEMISTRY
UNIVERSITY OF ILLINOIS DAVID SHAPIRO[3]
URBANA, ILLINOIS

RECEIVED SEPTEMBER 21, 1953

(3) On leave from the Weizmann Institute of Science, Rehovoth, Israel.

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.

G. D. SEARLE AND CO. R. M. DODSON
CHICAGO 80, ILLINOIS PAUL B. SOLLMAN
BYRON RIEGEL

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.

the rare earth ions were precipitated as oxalates and the amount of reaction was then determined by titration with standard alkali.

0.5 N La^{3+} depresses the rate of decarboxylation by 10%. In the presence of the smaller Y^{3+} ion the rate of decarboxylation is equal within the experimental error of 4–5% to the rate of reaction in the absence of rare earth. On the basis of the salt effect of these diamagnetic ions the dysprosium ion, which is slightly larger than the yttrium ion, would be expected to give rise to a slightly slower rate of decarboxylation than that in the absence of rare earth or in the presence of Y^{3+}. In three sets of experiments the rate of reaction in the presence of 0.5 N Dy^{3+} was found to be 8, 10 and 13% faster than in the presence of 0.5 N Y^{3+}. The observed acceleration is tentatively attributed to the paramagnetic character of the dysprosium ion.

Investigations of the effect of paramagnetic ions on the decarboxylation of natural and isotopically substituted compounds are being continued.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF CALIFORNIA
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KENNETH S. PITZER
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RECEIVED SEPTEMBER 28, 1953

SILICON TETRAFLUORIDE COMPLEX WITH ETHYLENEDIAMINE

Sir:

A recent report by C. J. Wilkins and D. K. Grant[1] of the preparation of two addition compounds of silicon tetrafluoride with one and with two molecules, respectively, of trimethylamine, has led us to add this note to the general subject of the coördinating power of the silicon halides. The fact that silicon assumes a coördination number of six in the fluosilicates, but is apparently unable to rise above four-coördination when chlorine atoms are employed as ligands, lends interest to the fact that in silicon tetrafluoride there is left sufficient room, as well as attractive force, about the silicon atom to accommodate one or even two molecules of trimethyl amine, with nitrogen the donor atom, as observed by Wilkins and Grant.

In following this general line of reasoning, we recently had prepared an addition compound of silicon tetrafluoride with ethylenediamine, the composition of which was determined by analysis and by molecular weight determinations as $SiF_4 \cdot CH_2NH_2NH_2CH_2$. No evidence of any other compound of the two reactants was obtained.

The ethylenediamine employed was twice redistilled from metallic sodium, and boiled within a range of 0.2°. The silicon tetrafluoride was prepared by thermal decomposition of precipitated barium fluosilicate, previously dried by heating at 200° for 3 hours in a vacuum of about 0.5 mm. The gas was preserved under pressure in a steel cylinder, and its properties agreed closely with the data given for pure silicon tetrafluoride by Jones, Kirby-Smith, Woltz and Nielson.[2]

(1) C. J. Wilkins and D. K. Grant, *J. Chem. Soc.*, 927 (1953).
(2) E. A. Jones, J. S. Kirby-Smith, P. J. H. Woltz and A. H. Nielson, *J. Chem. Phys.*, **19**, 242 (1951).

The reaction of the vapors of anhydrous ethylenediamine, introduced into an evacuated flask to the limit of its vapor pressure at room temperature with gaseous silicon tetrafluoride, subsequently added, resulted in the formation of the white, solid complex and in a reduction of the pressure in the flask. More ethylenediamine was then admitted, followed by more silicon tetrafluoride. The quantity of liquid diamine added was measured by means of a buret; the tetrafluoride was measured in a gas buret over mercury. In one run, repetition of the procedure yielded 8 g. of a light, fine, white powder which was dried at 130°C. and 0.5 mm. for 3 hr. to free it of any excess diamine. At 0.2 mm. pressure the substance is sublimable about 225°. In other runs, smaller quantities were prepared. *Anal.* Fluoride, calcd., for $SiF_4 \cdot CH_2NH_2NH_2CH_2$ 46.35; found, 46.65%. Nitrogen, calcd., 17.08, found, 15.96%.

It is clear that in this compound a chelate structure is present, with ethylenediamine occupying two of the six coördination positions about the silicon atom. It is of interest that further addition of the diamine, even if coördinated in unidentate fashion, is apparently excluded by spatial considerations.

DEPARTMENT OF CHEMISTRY
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RECEIVED SEPTEMBER 3, 1953

RATE OF THE ELECTRON-TRANSFER EXCHANGE REACTION BETWEEN MANGANATE AND PERMANGANATE IONS[1]

Sir:

We have successfully measured the rate of the isotopic exchange reaction between $MnO_4^=$ and MnO_4^-. Other investigators,[2,3,4] employing higher reactant and/or sodium hydroxide concentrations than we have used, had found complete exchange in contact times considerably longer than ours.

Using the separation procedure developed by Bonner and Potratz[4] of extracting MnO_4^- into a chloroform solution of triphenylsulfonium bromide, we have followed the exchange starting either with tagged $MnO_4^=$ or with tagged MnO_4^-. Separation methods involving the coprecipitation of MnO_4^- with tetraphenylarsonium perchlorate or perrhenate have also been used successfully. Reactions were started and stopped by the rapid ejection of solutions from automatic pipets into solutions being mechanically stirred.

Figure 1 shows three of our best exchange curves. In each of these runs the concentrations of $MnO_4^=$ and MnO_4^- were approximately equal; precise values for the concentrations of the individual reactants are not known however, because appreciable reduction of MnO_4^- to $MnO_4^=$ occurred. The dependence of the half-time of the exchange in 0.15 f NaOH at 1° on the total reactant concen-

(1) This work was supported by the National Science Foundation under grant G-196.
(2) W. F. Libby, THIS JOURNAL, **62**, 1930 (1940).
(3) H. C. Hornig, G. L. Zimmerman and W. F. Libby, *ibid.*, **72**, 3808 (1950).
(4) N. A. Bonner and H. A. Potratz, *ibid.*, **73**, 1845 (1951).

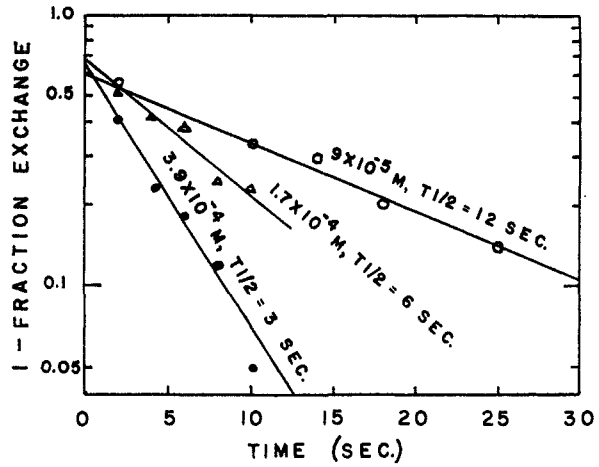

Fig. 1. Exchange curves 1°, 0.15*f* NaOH: concentrations are total manganese concentrations; $(C_6H_5)_3$ Br extraction separation used in the run at the intermediate concentration; $(C_6H_5)ReO_4$ coprecipitation separation used in the other two runs

tration is consistent with a second-order rate law with a constant of 650 M^{-1} sec.$^{-1}$.

In 2 *f* NaOH the rate of exchange is approximately twice that in 0.15 *f* NaOH, and the zero-time exchange is also greater. These observations are consistent with the nearly complete exchange in 15 seconds observed by Bonner and Potratz[4] for similar experimental conditions.

The fact that the rate of the exchange is small compared to collision frequencies shows that in aqueous solution the probability of electron transfer from $MnO_4^=$ to MnO_4^- is small, even though for these symmetrical reactants the Franck–Condon type restrictions are minimal.[5]

We hope to improve our technique sufficiently to make a detailed kinetic study of this exchange reaction.

(5) W. F. Libby, *J. Chem. Phys.*, **56**, 863 (1952).

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JOHN C. SHEPPARD
ARTHUR C. WAHL

RECEIVED SEPTEMBER 14, 1953

REFRACTIVE INCREMENT OF THYMUS NUCLEIC ACID

Sir:

Recently we have had occasion to question the numerical value (0.160 ml.-g.$^{-1}$) of the refractive increment, d*n*/d*c*, of thymus nucleic acid (DNA), as published by Tennent and Vilbrandt in THIS JOURNAL[1] and cited by Smith and Sheffer,[2] Katz,[3] and Doty and Bunce.[4] We have measured the d*n*/d*c* of two samples of DNA prepared in our laboratory from fresh thymus tissue. Sample No. 1 was prepared by the method of Mirsky and Pollister[5]; the nucleoprotein of sample No. 2 was prepared in the same way as sample No. 1, but was deproteinized using the dodecyl sulfate denaturation described by Marko and Butler.[6] The samples were dissolved in the desired salt solution, stirred for at least 24 hours, and then dialyzed for several days against the salt solution, the dialysate being replaced occasionally.

The difference Δn in index of refraction between the DNA solution and the last dialysate was in each case measured at 20°, using 4358 Å. light, by means of a differential refractometer. This instrument was somewhat similar to that described by Brice and Halwer[7] and was calibrated using data given by Stamm.[8] Using this calibration, a value of 0.178 ml.-g.$^{-1}$ was obtained for d*n*/d*c* of tobacco mosaic virus.

The concentration of each DNA sample was determined from its optical density at 2600Å. (measured by dilution into 0.1 *M* acetate buffer, *p*H 4.3), the ratio of density to phosphorus content for the sample and the per cent. phosphorus in DNA. For sample 1, the ratio of optical density (in 0.1 *M* acetate buffer, *p*H 4.3) in a 1-cm. cell to phosphorus was 6620 per mole of phosphorus/liter, and 6640 for sample 2. From data given by Sinsheimer and Koerner[9] on the nucleotide composition of DNA, it was computed that 9.35% by weight of the sodium salt of DNA is phosphorus.

Results are listed in the table:

DNA prepn.	Solvent	Δn	Concn. in g./ml.	$\Delta n/c$, ml.-g.$^{-1}$
1	0.1 *M* sodium acetate + 0.2 *M* sodium chloride, *p*H 5.7	0.701×10^{-4}	0.347×10^{-3}	0.202
	0.05 *M* sodium chloride	0.638×10^{-4}	0.323×10^{-3}	0.198
	0.001 *M* sodium chloride	0.599×10^{-4}	0.298×10^{-3}	0.201
2	0.1 *M* sodium acetate + 0.2 *M* sodium chloride, *p*H 5.7	1.63×10^{-4}	0.821×10^{-3}	0.199
	0.05 *M* sodium chloride	5.30×10^{-4}	2.62×10^{-3}	0.202
	0.001 *M* sodium chloride	1.68×10^{-4}	0.827×10^{-3}	0.204
			Av.	0.201

The combined effect on the observed Δn of Donnan equilibrium and of the binding of NaCl by the DNA was computed from data given by Shack, Jenkins and Thompsett[10] for the case where the solvent was 0.05 *M* NaCl. It was found that because of these two effects, the observed Δn of 5.30×10^{-4} may be too large by 1.9%. In making this calculation, it was necessary to assume that the polarizability of NaCl bound to the DNA is the same as that in a free solution of NaCl. For the other solvents the effect of salt binding and Donnan equilibrium cannot be calculated from the work of Shack, *et al.*, since they did not go to as low a NaCl concentration as 0.001 *M*, nor did they investigate the acetate buffered NaCl solution.

Since d*n*/d*c* enters to the second power in light-

(1) H. G. Tennent and C. F. Vilbrandt, THIS JOURNAL, **65**, 424 (1943).
(2) D. B. Smith and H. Sheffer, *Can. J. Research*, **B28**, 96 (1950).
(3) S. Katz, THIS JOURNAL, **74**, 2238 (1952).
(4) P. Doty and B. H. Bunce, *ibid.*, **74**, 5029 (1952).
(5) A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.*, **30**, 117 (1946).
(6) A. M. Marko and G. C. Butler, *J. Biol. Chem.*, **190**, 165 (1951).
(7) B. A. Brice and M. Halwer, *J. Opt. Soc. Am.*, **41**, 1033 (1951).
(8) R. F. Stamm, *ibid.*, **40**, 788 (1950).
(9) R. L. Sinsheimer and J. F. Koerner, *J. Biol. Chem.*, **198**, 293 (1952).
(10) J. Shack, R. J. Jenkins and J. M. Thompsett, *J. Biol. Chem.*, **198**, 85 (1952).

scattering determinations of molecular weights, the molecular weights of DNA as determined by Smith and Sheffer, Katz, and Doty and Bunce would seem to be too high by 37%. It should be pointed out that Tennent and Vilbrandt did not specify the wave length light used in their refractive measurements; hence direct comparison of their value of 0.160 with the above value of 0.201 should not be made.

DEPARTMENT OF PHYSICS
IOWA STATE COLLEGE
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T. G. NORTHROP
R. L. NUTTER
R. L. SINSHEIMER

RECEIVED JULY 24, 1953

VALINE BIOSYNTHESIS IN *TORULOPSIS UTILIS*[1]

Sir:

Results are reported herein which indicate that the carbon chain of lactic acid is a direct precursor of the valine carbon skeleton. The materials for this investigation were specimens of labeled valine isolated, by slight modifications of the method of Moore and Stein,[2] from hydrolysates of yeast grown in the presence of C^{14}-labeled tracer substances. Growth of the cells and other experimental details have been described previously.[3] Submission of the valines to a degradation procedure for radioactivity assay of each of the four different valine carbons gave the results shown in the table. Glycine, acetate, and lactate carboxyl carbons appeared only in the valine carboxyl; glycine and acetate α-carbons appeared approximately equally in all of the valine non-carboxyl carbons; and the lactate α-carbon appeared equally and nearly exclusively in carbons 2 and 3 of valine. The relatively low incorporation of acetate and glycine carbons precluded these substances, as well as citric acid cycle components, as direct precursors of valine. However, the relatively high incorporation of lactate carbons suggested that lactate or pyruvate may be the direct source of carbons for valine biosynthesis, and that acetate and glycine carbons were incorporated in valine *via* their prior conversion to pyruvate. The observed distribution of activity is in accord with the conversion of glycine to pyruvate *via* serine, and of acetate to pyruvate *via* the citric acid cycle and oxalacetate. If this postulation is correct, it follows that the methyl carbon of pyruvate should be the precursor of the valine methyl carbons. Indirect proof that carbon 3 of pyruvate can provide the carbon for the valine methyl carbons was obtained in the last experiment in the table in which it was found that carbon 1 of glucose, presumably *via* 3-labeled pyruvate, appeared preponderantly in the methyl carbons of valine.

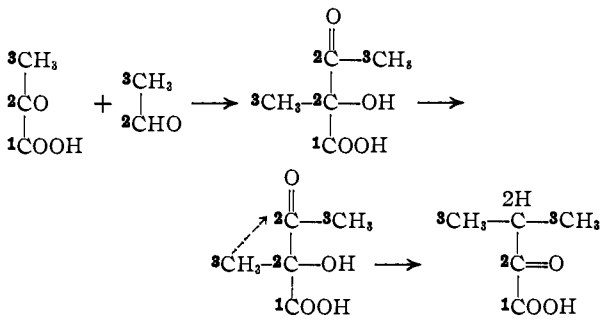
DISTRIBUTION OF LABELED CARBONS IN VALINE CARBON

Values are specific activities in cpm of $BaCO_3$, corrected for equal initial activities of substrates.

Valine carbon number[a]	Precursors: Acetate CH_3-	Acetate -COOH	Glycine $-CH_2$-	Glycine -COOH	Lactate -CHOH-	Lactate -COOH	Glucose-1-C^{14} -CHO
1	105	170	30	575	45	905	25
2	155	−3	365	−4	750	−5	45
3	150	0	310	0	700	} 5[b]	50
4,4'	155	0	335	0	20	}	358

[a] Numbering begins with valine carboxyl carbon. [b] Acetone not further degraded.

In speculating on the mechanism of this conversion, the equal incorporation of lactate carbon 2 into valine carbons 2 and 3 suggests a direct coupling of 2 lactate α-carbons. The only conceivable biological reaction of similar type is the condensation of pyruvate and acetaldehyde to yield acetolactic acid.[4] From the structure of this substance it is not unreasonable to assume that migration of a methyl group might occur, as in the pinacol or related rearrangements, to yield β,β'-dimethylpyruvic acid, a logical precursor of valine. Some precedent for the biological occurrence of methyl group migration has recently been provided by Woodward and Bloch.[5] This pathway is under further investigation.



LANKENAU HOSPITAL RESEARCH INSTITUTE
AND INSTITUTE FOR CANCER RESEARCH
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MURRAY STRASSMAN[6]
ALICE J. THOMAS
SIDNEY WEINHOUSE

RECEIVED AUGUST 14, 1953

(1) Aided by grants from the Atomic Energy Commission, contract No. AT(30-1)777; the American Cancer Society; and the National Cancer Institute of the Department of Health, Education and Welfare.

(2) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

(3) M. Strassman and S. Weinhouse, THIS JOURNAL, **74**, 1726 (1952).

(4) T. P. Singer and J. Pensky, *Biochim. et Biophys. Acta*, **9**, 316 (1952).

(5) R. B. Woodward and K. Bloch, THIS JOURNAL, **75**, 2023 (1953).

(6) Postdoctoral Fellow of the National Institutes of Health, Department of Health, Education and Welfare.

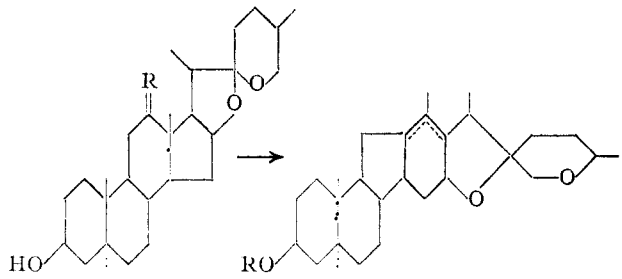
REARRANGEMENT OF THE STEROID C/D RINGS. SYNTHESIS OF AN 11-KETO-$\Delta^{13(17a)}$-C-*NOR*/D-*HOMO*-STEROID

Sir:

Hecogenin (I) in the form of its toluene *p*-sulfonylhydrazone derivative (Ia), m.p. 259–60° (dec.); found: S, 5.39; $\lambda_{max.}^{CH_3OH}$ 226 mμ (4.1), was submitted to a Bamford–Stevens rearrangement[1] with sodium in ethylene glycol to yield the C-*nor*/D-*homo*-sapogenin (II) m.p. *ca.* 110°; found: C, 77.95; H, 10.00. *Acetate* (IIa) m.p. 142–144°; $[\alpha]^{23}D$ −52.6 ($CHCl_3$). Found: C, 76.03; H, 9.72. II was found to be identical with a companion olefin isolated together with III from the solvolytic rearrangement of the rockogenin derivative (IV)[2]; II was also formed in good yield from III on treatment of the latter with formic acid at room temperature. The endocyclic olefin (II)

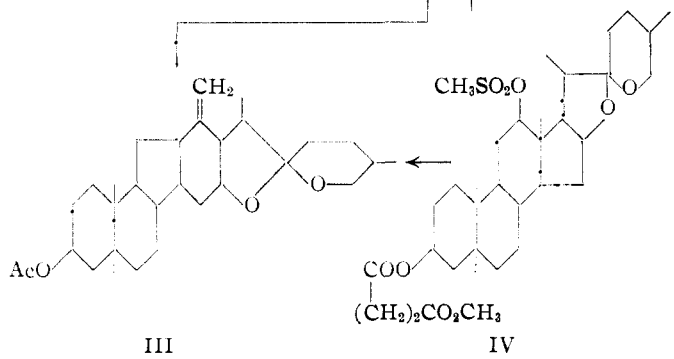
(1) W. R. Bamford and T. S. Stevens, *J. Chem. Soc.*, 4735 (1952).

(2) R. Hirschmann, C. S. Snoddy, Jr., and N. L. Wendler, THIS JOURNAL, **74**, 2693 (1952).

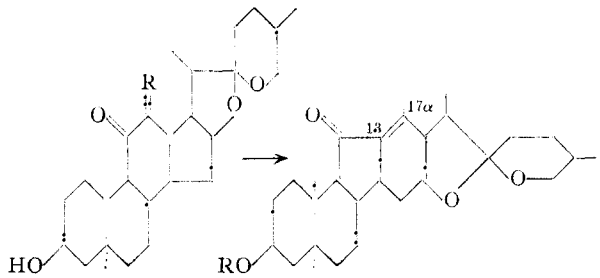


I, R, O =
Ia, R, $C_7H_7SO_2NHN$ =

II, R, H
IIa, R, CH_3CO



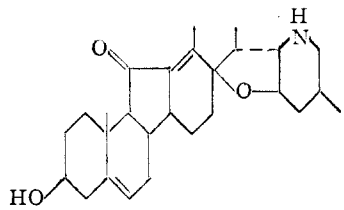
III IV



V, R, O =
Va, R, $C_7H_7SO_2NHN$ =

VI, R, H
VIa, R, CH_3CO

exhibited no absorption in the double bond region of the infrared and was smoothly converted with



VII

osmium tetroxide to a triol which on treatment with acetic anhydride in pyridine at room temperature gave only a monoacetate derivative m.p. 215–18°; $[\alpha]^{25}D$ −39.3° ($CHCl_3$). Found: C, 71.00; H, 9.45.

In a similar manner 11-ketohecogenin (V)[3] was converted to its toluene *p*-sulfonylhydrazone derivative (Va), m.p. 156–158° (dec.); $\lambda\lambda_{max.}^{CH_3OH}$ 228 mμ (3.90), 275 mμ (3.82). Found: N, 4.84. The latter rearranged on treatment with potassium hydroxide in refluxing ethylene glycol to give the 11-keto-$\Delta^{13(17a)}$-C-*nor*/D-*homo*-sapogenin (VI) m.p. 190–192°; $[\alpha]^{24}D$ −78.4° ($CHCl_3$); $\lambda\lambda_{max.}^{CH_3OH}$ ultraviolet 255 mμ (4.17), 350 mμ (2.88); $\lambda\lambda_{max.}^{CHCl_3}$ infrared 5.85 μ, 6.1 μ (more intense). Found: C, 75.54; H, 9.62. *Acetate:* (VIa) m.p. 178.5–179.5°; $[\alpha]^{24}D$ −80.7° ($CHCl_3$); $\lambda\lambda_{max.}^{CH_3OH}$ 225 mμ (4.18), 350 mμ (2.87). Found: C, 73.74; H, 8.92.

The spectral characteristics of VI are essentially the same as those of jervine (VII).[4] Of particular note is the unique reversal in intensity of the C=O and C=C bands in the infrared spectra of both VI and jervine.

(3) C. Djerassi, H. Ringold and G. Rosenkranz, *ibid.*, **73**, 1513 (1951).

(4) J. Fried, O. Wintersteiner, M. Moore, B. M. Iselin and A. Klingsberg, *ibid.*, **73**, 2970 (1951).

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RECEIVED SEPTEMBER 14, 1953

BOOK REVIEWS

Computing Methods and the Phase Problem in X-Ray Crystal Analysis. Report of a Conference Held at The Pennsylvania State College, April 6–8, 1950. By RAY PEPINSKY (Editor). The X-Ray Crystal Analysis Laboratory, Department of Physics, The Pennsylvania State College, State College, Pa. 1952. xvii + 390 pp. 21.5 × 27.5 cm. Price, $7.50.

The conference report presented in this volume is the result of a meeting of about fifty specialists in the field of crystal structure determination from X-ray diffraction data, which was organized by Professor Ray Pepinsky at the Pennsylvania State College under the joint sponsorship of the Rockefeller Foundation and the Office of Naval Research. It was the intent of the conference and the resulting report to review the current status of the problem posed by the lack of experimental information on the phases of the X-ray beams scattered by a given crystal and also of the progress in computing methods applicable to crystal structure determination. This intent has been very successfully fulfilled, and this collected review is a basic contribution to the literature of that field.

The report consists essentially of four parts. The first is a group of ten papers dealing with the basic mathematical problems involved in the application of Fourier transformations in the analysis of the experimental intensity data. Following an introduction by R. Pepinsky and a general statement of the problem by J. M. Bijvoet, papers by C. A. Beevers and A. L. Patterson discuss the structural information which can be obtained from the "Patterson" synthesis using $|F|^2$ coefficients. M. J. Buerger then presents an-