TABLE I

EFFECT OF HEATING "DPN ISOMER" ON GROWTH OF NICOTINAMIDE REQUIRING NEUROSPORA MUTANT

0.04 of a micromole of each pyridine compound was added in a volume of 20 ml.; growth was for a period of three days.

Additions	Dry weight of mat, milligrams
None	0
Nicotinamide	107
DPN	73
DPN Heated[a]	103
"DPN Isomer"	0
"DPN Isomer" heated[a]	100

[a] Heated at 100° for 30 minutes. No reaction with cyanide occurred after heating, indicating complete cleavage of the N-glycosidic linkage.

(with an absorption maximum at 322 mμ.) Nicotinamide mononucleotide obtained from DPN has a maximum cyanide absorption at 325 mμ. The two mononucleotides also react at different rates with a number of different nucleotidases. Heating the two mononucleotides yields pentose phosphate products which are identical with ribose 5'-phosphate as determined by chemical and chromatographic procedures. The ribose phosphates also react at identical rates in the pentose isomerase and transketolase reactions of Horecker, *et al.*[6]

The fact that the isomer appeared to be different from "normal" DPN in the nicotinamide glycosidic linkage, led us to carry out optical rotation measurements of the mononucleotides. As can be seen from Table II, the two mononucleotides have completely different rotations. The isomer mononucleotide is positive rotating whereas the mononucleotide of DPN is negative rotating.[7] The difference is also observed in the dinucleotide form; the isomer has a rotation of +14° whereas DPN has a rotation of −35°. Such rotations might be expected since 5'-AMP has a negative rotation of about −40°. Heating the two mononucleotides yields products which give only a very slight rotation and which are quite similar. We therefore feel that the isomer contains nicotinamide riboside in the α position as contrasted to the beta nicotinamide ribosidic linkage of DPN.

TABLE II

OPTICAL ROTATIONS OF DPN AND DERIVATIVES

	Compound	$[\alpha]^{23}D$ 1% in H_2O
(1)	DPN	−34.8
(2)	DPN isomer	+14.3
(3)	Nicotinamide mononucleotide from (1)	−38.3
(4)	Nicotinamide mononucleotide from (2)	+58.2
(5)	Ribose 5'-phosphate from (3)	− 2.7
(6)	Ribose 5'-phosphate from (4)	− 4.7
(7)	5'-Adenylic acid	−40.0

We cannot as yet state with certainty whether the isomer is formed during isolation or is a naturally occurring product. However, we have been able to detect the isomer in crude extracts from yeast and animal tissues, by treating the crude extracts with the *Neurospora* DPNase and then assaying for cyanide reacting material. It is of interest to note that the α isomer appears to have a somewhat more negative potential (closer to the hydrogen electrode at *p*H 7) than the beta isomer of DPN.[8] We are now investigating the possible biological significance of the compound. Details of the properties of the α isomer of DPN will be presented elsewhere.[9]

(6) B. L. Horecker, P. Z. Smyrniotis and H. Klenow, *J. Biol. Chem.*, **205**, 661 (1953).

(7) The values given in Table II have an accuracy of ±2 to 3°. The 5'-AMP present in both DPN and the isomer is of the beta glycosidic linkage.

(8) M. M. Weber and N. O. Kaplan, unpublished experiments. The potential was estimated from the end-point of an electron exchange reaction between the reduced isomer and oxidized DPN, catalyzed by a *Clostridial* enzyme.

(9) We wish to thank Dr. Joseph Riden for help in the optical rotation studies and Dr. B. L. Horecker for carrying out the transketolase test.

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STUDIES ON THE BIOSYNTHESIS OF ARTERENOL. ENZYMATIC DECARBOXYLATION OF DIASTEREOISOMERS OF HYDROXYPHENYLSERINES[1]

Sir:

Conflicting reports regarding the decarboxylation of 3,4-dihydroxyphenylserine (DOPS) by tissue extracts *in vitro* have appeared[2-7] although studies *in vivo*[7,8] point to the formation of arterenol. The availability of the diastereoisomers of DOPS,[9] *m*-hydroxyphenylserine (MOPS)[10] and *p*-hydroxyphenylserine (POPS),[10] and of a chromatographic method for assessing their homogeneity[11] have allowed reëvaluation of their activity.

erythro-DOPS is decarboxylated at an appreciable rate by hog kidney enzyme,[12] but to a lesser extent than DOPA, and *threo*-DOPS and *erythro*-MOPS are decarboxylated rather slowly (Table I). However, *threo*-DOPS and *erythro*-DOPS are decarboxylated at the same rate by whole liver homogenate.

Arterenol (R_F 0.45) and α-aminomethyl-*m*-hydroxybenzyl alcohol (R_F 0.62), respectively,

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(2) H. Blaschko, P. Holton and G. H. Sloane Stanley, *Brit. J. Pharmacol.*, **3**, 315 (1948).

(3) K. H. Beyer, H. Blaschko, J. H. Burn and H. Langemann, *Nature*, **165**, 926 (1950).

(4) H. Blaschko, J. H. Burn and H. Langemann, *Brit. J. Pharmacol.*, **5**, 431 (1950).

(5) T. Sourkes, P. Heneage and Y. Trano, *Arch. Biochem. Biophys.*, **40**, 185 (1952).

(6) G. Fodor and J. Kiss, *Acta Univ. Szeged., Chem. et Phys.*, **3**, 26 (1950).

(7) K. H. Beyer, "Advances in Chem. Ser.," No. **2**, 37 (1950).

(8) C. G. Schmiterlöw, *Brit. J. Pharmacol.*, **6**, 127 (1951).

(9) W. A. Bolhofer, THIS JOURNAL, **76**, 1322 (1954).

(10) W. A. Bolhofer, *ibid.*, **75**, 4469 (1953).

(11) W. Drell, unpublished.

(12) O. Schales and S. S. Schales, *Arch. Biochem.*, **24**, 83 (1949).

TABLE I

ENZYMATIC DECARBOXYLATION OF PHENYLSERINES

Each Warburg flask contained 20 micromoles DL-substrate in one sidearm, 0.2 ml. 3 N H_2SO_4 in the other sidearm, lyophilized hog kidney extract or homogenized rat liver (wet wt.), 2.0 ml. 0.1 M phosphate buffer at pH 6.8, 80 micrograms of calcium pyridoxal-5-phosphate, and water to 4.5 ml. in the main compartment; gas, nitrogen; temp., 37°; time, 1–1.5 hr. The acid was tilted in at the end of the run to expel retained CO_2.

	Hog Kidney Extract						Rat Liver Homogenate		
Expt. No.	1	2	3	4	5	6	7	8	9
Mg. enzyme	15	15	30	30	30	30	100	150	150
	Microliters of CO_2 evolved								
DL-*erythro*-DOPS	60	61	79	54	54	58	21	27	47
DL-*threo*-DOPS	16	19				0	27	53	44
DL-*erythro*-MOPS	26	27	21	23					
DL-*threo*-MOPS	0	4							
DL-*threo*-POPS	8	2							
DL-DOPA	169	162			174		80		179

were identified in the flask contents by paper chromatographic techniques [solvent: 2-propanol, 70; acetic acid, 5; water, 25; descending method; spray reagents: (1) potassium ferricyanide–ferric sulfate[13,14]; (2) N,2,6-trichloro-p-benzoquinone imine[15]] after treatment of *erythro*-DOPS (R_F 0.25) and *erythro*-MOPS (R_F 0.40) with hog kidney extract at four times the amounts of reactants shown in Table I in a Dubnoff incubator under nitrogen, followed by deproteinization and lyophilization. Arterenol was similarly identified after treatment of either *erythro*- or *threo*-DOPS (R_F 0.17) by rat liver homogenate (experiments 8 and 9 of Table I combined). After treatment of DL-*erythro*-DOPS (110 mg.) with hog kidney extract (360 mg.), fractionation with a buffered Amberlite IRC-50 column[16] showed the presence of (−)-*erythro*-DOPS in the unabsorbed effluent after concentration [R_F 0.25; observed α_D −0.3°, c = 0.16% (in 3N HCl) by chemical analysis,[17] l = 4 dm.] and of (+)-arterenol in the acid effluent [R_F 0.45; observed ratio of concentrations in mg. per ml. by bioassay (pithed cat blood pressure rise) and chemical assay[17] was 0.15/1.6 or 0.09; this ratio was 0.02 for (+)-arterenol and 1.00 for (−)-arterenol].

The phenylserine derivatives were compared with L- and DL-DOPA in cocainized pithed rat and cat preparations.[18] The relative activities in terms of the systolic blood pressure increases due to the pressor amines liberated by decarboxylation *in vivo*[19] are summarized in Table II.

The arterenol produced by rats injected with *erythro*- and *threo*-DOPS (25 mg./kg. I.V.) was isolated by treating the urine with alumina,[20] dissolving the alumina in acid, concentrating, streaking on paper, developing with the 2-propanol–acetic acid–water solvent, and eluting the area corresponding to the arterenol R_F. Preliminary data from comparison of chemical[17] and biological assays suggest that the arterenol from *threo*-DOPS is the natural biologically active isomer (*cf.* ref. 2), while that produced from *erythro*-DOPS is the relatively inactive optical antipode.

(13) W. O. James, *Nature*, **161**, 851 (1948).

(14) M. Goldenberg, M. Faber, E. J. Alston and E. C. Chargaff, *Science*, **109**, 534 (1949).

(15) H. G. Bray, W. V. Thorpe and K. White, *Biochem. J.*, **46**, 271 (1950).

(16) S. Bergström and G. Hansson, *Acta Physiol. Scand.*, **22**, 87 (1951).

(17) U. S. von Euler and U. Hamberg, *Science*, **110**, 561 (1949).

(18) R. S. Pogrund and W. G. Clark, unpublished.

(19) The absence of pressor amines in the amino acids was checked by biological assay of the materials before and after passage through a small Amberlite IRC-50 column buffered at pH 6.5,[16] a procedure which was shown to remove these amines quantitatively if present.

(20) M. Goldenberg, I. Serlin, T. Edwards and M. M. Rapport, *Am. J. Med.*, **16**, 310 (1954).

TABLE II

BLOOD PRESSURE EFFECTS OF PHENYLSERINES

	I.V. dose (mg./kg.)	B.P. respons (rel. value)
DL-DOPA	5–10	1.00
DL-*erythro*-DOPS	10–50	0.11
DL-*threo*-DOPS	10–50	0.55
DL-*erythro*-MOPS	50	0.04
DL-*threo*-MOPS	50	<0.02
DL-*threo*-POPS	50	0.08
DL-*erythro*-phenylserine	50	0.03

Because of the low pressor activity of (+)-arterenol formed from *erythro*-DOPS and of 3,4-dihydroxyphenethylamine formed from DOPA compared to the high activity of (−)-arterenol formed from *threo*-DOPS, the relative blood pressure responses shown in Table II do not indicate the relative amount of decarboxylation of the above amino acids.

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TOTAL SYNTHESIS OF TESTOSTERONE

Sir:

Formal total syntheses of testosterone, employing naturally derived intermediates as relays, have already been described.[1] We now wish to report a direct approach which has afforded for the first time totally synthetic testosterone (VI) in form *dl*.

The readily available tetracyclic ketone I[1c] was converted to the ethylene ketal II, m.p. 102–103.2°

(1) See for example (*a*) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, THIS JOURNAL, **73**, 2403, 3547 (1951), and **74**, 4223 (1952); (*b*) H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, H. Holtermann and R. Robinson, *Chemistry and Industry*, 389 (1951), and *J. Chem. Soc.*, 361 (1953); (*c*) W. S. Johnson, B. Bannister, B. M. Bloom, A. D. Kemp, R. Pappo, E. R. Rogier and J. Szmuszkovicz, THIS JOURNAL, **75**, 2275 (1953); (*d*) A. L. Wilds, J. W. Ralls, D. A. Tyner, R. Daniels, S. Kraychy and M. Harnik, *ibid.*, **75**, 4878 (1953).