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

(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

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.

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.

VETERANS ADMIN. CENTER AND MEDICAL CENTER
UNIVERSITY OF CALIFORNIA
LOS ANGELES, CALIFORNIA

W. J. HARTMAN
R. S. POGRUND
W. DRELL[21]
W. G. CLARK

RECEIVED OCTOBER 22, 1954

(21) Established Investigator of the American Heart Association.

TOTAL SYNTHESIS OF TESTOSTERONE

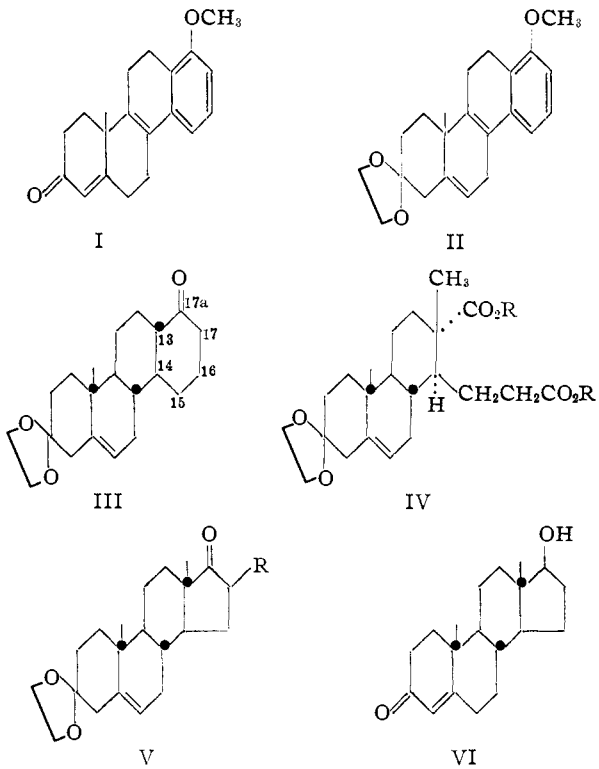
Sir:

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).

or 119–119.7°, $\lambda_{max}^{alc.}$ 220 mμ (log ε 4.44), 266 (4.08), 272 (4.04) (Found: C, 78.2; H, 8.17), which on vigorous reduction with lithium and alcohol (40%) in ammonia,[1c,2,3] followed by selective hydrolysis of the enol ether group with dilute aqueous methanolic oxalic acid and then treatment with sodium acetate in ethanol to effect isomerization of the unsaturated ketones to the conjugated isomers gave a mixture of the (preponderant) 13,14-dehydroketone III (C=C at 13,14), m.p. 142.8–143.2° $\lambda_{max}^{alc.}$ 246.5 mμ (log ε 4.13) (Found: 76.9; H, 8.68) and the 16,17-dehydroisomer III (C=C at 16,17), m.p. 175–176.5°, $\lambda_{max}^{alc.}$ 225 mμ (log ε 3.94) (Found: C, 76.9; H, 8.55) separable by chromatography.[4] Hydrogenation over palla-



dium-on-carbon of both of these isomers (the former in the presence of a trace of potassium hydroxide) proceeded selectively to give the ketal III of *dl*-18-nor-D-homoandrostenedione, m.p. 142–143° (Found: C, 76.7; H, 9.43). Conversion to the furfurylidene derivative, m.p. 187–188.5° (Found: C, 76.2; H, 7.89) followed by methylation[5] afforded the ketal of *dl*-17-furfurylidene-D-homoandrostenedione, m.p. 210–211° (Found: C, 76.7; H, 8.16) along with the 13-iso (preponderant) compound, m.p. 187–188.5° (Found: C, 76.7; H, 8.18). Ozonolysis of these angularly methyl-

(2) W. S. Johnson, R. Pappo and A. D. Kemp, THIS JOURNAL, **76**, 3353 (1954).

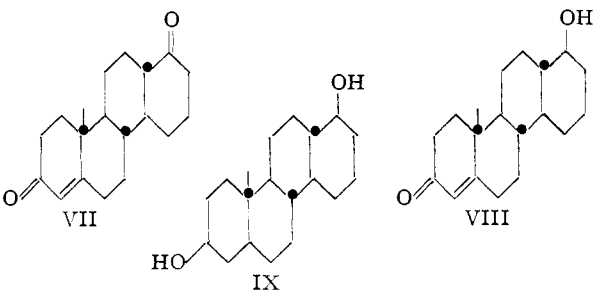
(3) Under milder (10% alcohol) conditions only the styrene double bond was reduced giving predominantly the dihydro ketal with the *anti-trans* configuration, m.p. 150–150.2° (Found: C, 77.7; H, 8.39). Acid-catalyzed hydrolysis yielded the α,β-unsaturated ketone, m.p. 154.5–155.5°, $\lambda_{max}^{alc.}$ max. 232 mμ (log ε 4.30), 278 (3.22) (Found: C, 81.3; H, 8.19).

(4) *Cf.* The products similarly formed in other series, refs. 1c and 2.

(5) *Cf.* W. S. Johnson, THIS JOURNAL, **65**, 1317 (1943).

ated C_{13} epimers did not proceed satisfactorily, but prolonged treatment with methanolic alkaline hydrogen peroxide effected selective oxidation to produce the corresponding etiohomobilienic acid derivative IV (R = H). Esterification with diazomethane gave IV (R = CH_3), m.p. 162.5–163.5° (Found: C, 68.5; H, 8.65) and 13-iso IV (R = CH_3), m.p. 122–123° (Found: C, 68.6; H, 8.55). Treatment of the former with alcohol-free potassium *t*-butoxide in benzene effected Dieckmann cyclization to give a keto ester, which on heating in boiling *p*-cymene decomposed to give the 3-ketal V (R = H) of *dl*-androstenedione, m.p. 167–169° (Found: C, 76.2; H, 9.06), having an infrared spectrum identical with that of the naturally derived *d*-compound. Reduction of V (R = H) with sodium borohydride gave *dl*-testosterone-3-ketal, m.p. 180–181.5° (Found: C, 75.9; H, 9.63), infrared spectrum identical with that of naturally derived *d*-compound. Acid hydrolysis afforded *dl*-testosterone (VI), m.p. 167.5–169° (Found: C, 79.1; H, 9.69) having an infrared spectrum indistinguishable from that of naturally derived *d*-testosterone.

In view of the relatively high androgenic activity of *dl*-18-nor-D-homoandrostane-3,17a-dione[6] it was considered of interest to prepare other 18-nor-D-homo steroids for physiological examination. Acid hydrolysis of the ketal III produced *dl*-18-nor-D-homoandrostenedione (VII), m.p. 146.5–147.5°, $\lambda_{max}^{alc.}$ 240 mμ (log ε 4.2) (Found: C, 79.5; H, 9.14).



Preliminary assays in rats performed by Drs. R. K. Meyer and Elva G. Shipley of the Department of Zoology indicate that this *dl* substance possesses about one-half of the androgenic activity of, and approximately the same myotrophic activity as, *d*-androstenedione. Reduction of the ketal III with lithium and alcohol in ammonia and acid hydrolysis of the product gave *dl*-18-nor-D-homotestosterone (VIII), m.p. 172–173°; acetate, m.p. 182–183° (Found: C, 76.6; H, 9.38); propionate 149.5–150.5° (Found: C, 76.8; H, 9.41). Physiological tests indicate that *dl*-VIII has very little androgenic or myotrophic activity, but the acetate and the propionate are about one-tenth as active as the corresponding derivatives of *d*-testosterone in the myotrophic test and about one-thirtieth as measured by the ventral prostate.

Reduction of VII with lithium and alcohol in ammonia gave *dl*-18-nor-D-homoandrostane-3β,17aβ-diol (IX), m.p. 210–211° (Found: C, 77.8; H, 10.91); diacetate, m.p. 169.5–170° (Found: C, 73.5; H, 9.67). This substance is

(6) W. S. Johnson, H. Lemaire and R. Pappo, *ibid.*, **75**, 4866 (1953).

identical with material obtained by similar reduction of *dl*-18-nor-D-homoepiandrosterone[1c] and is also readily prepared by direct reduction of the 13,14-dehydro precursors. *dl*-IX is devoid of significant androgenic activity, but exhibits about one-fifteenth of the myotrophic activity of *d*-testosterone.

We are grateful to the National Science Foundation, the Research Committee of the Graduate School of the University of Wisconsin, the G. D. Searle & Co., and the Sterling-Winthrop Research Institute for giving support to this program.

WILLIAM S. JOHNSON
BRIAN BANNISTER
RAPHAEL PAPPO
J. E. PIKE
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

RECEIVED DECEMBER 27, 1954

CARBAMYL PHOSPHATE, THE CARBAMYL DONOR IN ENZYMATIC CITRULLINE SYNTHESIS[1]

Sir:

The recent work on a phosphorolysis of citrulline in microbial extracts by Knivett,[2] by Slade[3,4] and by Korzenovsky and Werkman[5,6] and more recently by Stulberg and Boyer[7] has greatly advanced the understanding of the mechanism of this reaction. It seemed of considerable promise now to attempt the identification of the probable phosphorylated intermediary in this system which appeared to have a certain similarity to the so-called phosphoroclastic reaction of pyruvate in microbial extracts. In attempts to identify a phosphorylated intermediary, using extracts of *Streptococcus fecaelis* R, no reaction between ATP[8] and ornithine could be observed. As shown in Table I, however, on incubation of an equilibrium mixture of ammonium carbonate-carbamate with ATP, or better, phosphopyruvate + ADP, a relatively stable phosphorylated compound was formed. The compound decomposes only slowly in the Fiske and SubbaRow phosphate reagent, but hydrolyzed completely on one minute heating with 0.01 normal hydrochloric acid to 100° and is determined in this manner.

This precursor of the carbamyl group in citrulline has been identified by synthesis as carbamyl phosphate. Carbamyl phosphate is surprisingly easily prepared by mixing dihydrogen phosphate with cyanate in the following manner: 0.1 mole of potassium dihydrogen phosphate and 0.1 mole of potassium cyanate were dissolved in 100 milliliters of water, the solution warmed to 30° for 30 minutes, and then cooled in ice. To the cool solution, an ice-cold solution of 0.3 mole of lithium hydroxide and 0.2 mole of perchloric acid in 83 milliliters of water were added slowly, final *p*H 8.3. A precipitate forms which consists of potassium perchlorate and lithium phosphate. This is removed by filtration. The supernate contains the lithium carbamyl phosphate. This is precipitated by slow addition of an approximately equal volume of ethanol. On reprecipitation with ethanol, dilithium carbamyl phosphate of a purity of 90 to 95 per cent. was obtained which was used for enzymatic tests.

TABLE I

FORMATION OF CARBAMYL PHOSPHATE FROM ATP

The complete incubation mixture for the formation of carbamyl phosphate consisted of: 200 μM. tris-(hydroxymethyl)-aminomethane buffer, *p*H 8.5; 5 μM. $MgCl_2$; 25 μM. KF; 0.6 μM. of ADP, *p*H 7.0; 100 μM. ammonium carbonate; 5.1 μM. phosphoenol pyruvate; 10 μM. L-ornithine; 0.01 mg. crystalline pyruvate kinase; and 0.5 mg./ml. of *Streptococcus fecaelis* extract in 1 ml. final volume. Vessels were incubated at 30° for 30 minutes. Carbamyl phosphate is that phosphorus which is hydrolyzed by 0.01 *N* HCl in 1 minute at 100°. Citrulline was determined according to Archibald.[9]

		P_i, μM./ml.	P_u, μM./ml.	Citrulline, μM./ml.
1	No enzyme	0.20	0.41[a]	0
2	No phosphoenol pyruvate	0.15	0.05	0
3	No Mg or ornithine	0.32	0.46[a]	0
4	No ornithine	0.45	1.30	0
5	Complete	5.50	0.10	5.1

[a] This blank shows that our hydrolysis procedure decomposes a small fraction of the phosphoenol pyruvate.

The synthetic compound behaved analogously to the enzymatically formed compound with regard to acid hydrolysis and relative stability in the Fiske–SubbaRow molybdate mixture. Citrulline formation from synthetic carbamyl phosphate and ornithine with the microbial enzyme are shown in Table II. It may be noted that a small part of the compound decomposed spontaneously in the absence of enzyme or in its presence if ornithine is omitted. From observations of the previous workers on the phosphorolytic split of citrulline in the presence of ADP with the formation of ATP, the intermediary was expected to react easily with ADP. This is confirmed in the experiment shown in Table III, which shows a rapid reaction. We therefore formulate citrulline synthesis as

$$H_2N\cdot\overset{\overset{O}{\|}}{C}\cdot OH + ADP\cdot O\sim PO_3^- \longleftrightarrow H_2N\cdot\overset{\overset{O}{\|}}{C}\cdot O\sim PO_3^- + ADP \quad (1)$$

$$H_2N\cdot\overset{\overset{O}{\|}}{C}\sim O\cdot PO_3^- + NH_2\cdot(CH_2)_3\cdot CHNH_2\cdot COOH \longleftrightarrow H_2N\cdot\overset{\overset{O}{\|}}{C}\sim NH\cdot(CH_2)_3\cdot CHNH_2\cdot COOH + HO\cdot PO_3^- \quad (2)$$

Magnesium ion is required in reaction (1) but not in (2) (*cf.* Tables III and II).

Experiments with mitochondria have shown that CAP in the animal system likewise donates carbamyl to ornithine. The carbamyl-ornithine kinase appears more stable and far more active than the over-all reaction starting with ATP. In the

(1) This investigation was supported by research grants from the Cancer Institute of the National Institutes of Health, Public Health Service and the Life Insurance Medical Research Fund.

(2) V. A. Knivett, *Biochem. J.*, **50**, XXX (1952); **58**, 480 (1954).

(3) H. D. Slade and W. C. Slamp, *J. Bact.*, **64**, 455 (1952).

(4) H. D. Slade, *Arch. Biochem. Biophys.*, **42**, 204 (1953).

(5) M. Korzenovsky and C. H. Werkman, *ibid.*, **41**, 233 (1952).

(6) M. Korzenovsky and C. H. Werkman, *Biochem. J.*, **57**, 343 (1954).

(7) M. P. Stulberg and P. D. Boyer, THIS JOURNAL, **76**, 5569 (1954).

(8) The following abbreviations are used: ATP for adenosine triphosphate; ADP, adenosine diphosphate; CAP, carbamyl phosphate; P_i, orthophosphate; P_u, unstable phosphate; and P_{10}, phosphate hydrolyzed in 10 minutes with *N* HCl at 100°.

(9) R. M. Archibald, *J. Biol. Chem.*, **156**, 121 (1954).