striking separation of properties has now been demonstrated in certain C-16 halogenated estrone derivatives. Some of these show lipid-shifting to feminizing ratios about one hundred times that of estrone as, for example, 16α-chloroestrone methyl ether (I) and 16α-iodoestrone methyl ether (II).

The enol acetate of estrone methyl ether[1] in carbon tetrachloride was treated with chlorine and potassium carbonate to give I, m.p. 177–179°; $[\alpha]_D$ +161°;[2] (*Anal.* Calcd. for $C_{19}H_{23}ClO_2$: C, 71.57; H, 7.27; Cl, 11.12. Found: C, 71.53; H, 7.59; Cl, 11.17). To establish the configuration of the chlorine atom I was reduced with lithium aluminum hydride,[3] yielding 16α-chloroestradiol 3-methyl ether (III), m.p. 112–114°; $[\alpha]_D$ +72.5°; (*Anal.* Calcd. for $C_{19}H_{25}ClO_2$: C, 71.12; H, 7.85. Found: C, 71.40; H, 7.71) and 16α-chloroepiestradiol 3-methyl ether (IV), m.p. 162–164°; $[\alpha]_D$ +68.4°; (*Anal.* Found: C, 71.39; H, 7.80). Chromic acid oxidation converted both III and IV again into the ketone I. Treatment with alcoholic 3-methoxy-potassium hydroxide rearranged the *cis* chlorohydrin, IV, to estrone 3-methyl ether; similar treatment converted III into 16β,17β-epoxy-1,3,5-(10)-estratriene, m.p. 116–117°, $[\alpha]_D$ +115° (*Anal.* Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.04; H, 8.75) whose structure was confirmed by reduction with lithium aluminum hydride to 3-methoxy-16β-hydroxy-1,3,5(10)-estratriene, m.p. 105–107°, identical with an anthentic sample.[4]

Iodination[5] of the enol acetate yielded II, m.p. 161–166°; $[\alpha]_D$ +91°; (*Anal.* Calcd. for $C_{19}H_{23}IO_2$: C, 55.62; H, 5.65; I, 30.93. Found: C, 55.71; H, 6.04; I, 30.27) and 16β-iodoestrone 3-methyl ether (VI), m.p. 163–166°; $[\alpha]_D$ +178°; (Found: C, 55.43; H, 5.73; I, 30.48). Configurational assignments for II and IV were made relative to molecular rotational differences given for the 16-bromoandrostan-17-ones.[3]

TABLE I

LIPID-SHIFTING AND ESTROGENIC POTENCIES

Compound	N[a]	Lipid potency (A)	N[b]	Estrogen potency (B)	Ratio (A/B)
Estrone	95	100 (standard)	28	100 (standard)	1
I	39	90	17	0.79	114 (86–151)[c]
VII	11	22	6	1.3	20 (12.5–32.0)
II	24	143	10	1.8	95 (65–139)

[a] Total number of animals used at a minimum of three dose levels employed in calculation of lipid-shifting potency. [b] Number of groups of 8–10 mice employed in calculation of estrogenic potency. [c] Numbers in parentheses represent limits within which the true value has a 95% probability of being found.

This series of halides, including 16α-bromoestrone 3-methyl ether (VII),[1,6] was tested for lipid-shifting and feminizing effects. An index of the former was the reduction in cholesterol-phospholipid ratio observed in a 3-day test with cholesterol-fed cockerels.[7] Uterine growth in intact, immature mice was used to estimate feminizing activity.[8] Potencies in these tests relative to the estrone standard are shown in the table. The ratio A/B is a measure of the separation of the two kinds of activity[9] and presumably will give some indication of therapeutic efficiency. The more potent members of this series are active on oral administration.

(1) W. S. Johnson and W. F. Johns, THIS JOURNAL, **79**, 2005 (1957).

(2) Analytical data was obtained by Dr. R. T. Dillon and the Analytical Department. All rotations were measured in chloroform; melting points were taken on the Kofler hot stage.

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(4) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **213**, 343 (1955). We wish to thank Dr. D. A. Tyner for kindly providing this material.

(5) *Cf.* C. Djerassi and C. T. Lenk, THIS JOURNAL, **76**, 1724 (1954).

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(9) We wish to thank David W. Calhoun for statistical consultations.

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THE STEPWISE DEGRADATION OF THYMIDINE OLIGONUCLEOTIDES BY SNAKE VENOM AND SPLEEN PHOSPHODIESTERASES

Sir:

The chemical polymerization[1] of thymidine 5-phosphate (TP) yields a number of 5′ → 3′ linked oligonucleotides[2] (T_nP_n), which bear 5′-phosphate end-groups. Using these compounds and the corresponding series (T_nP_{n-1}) obtained by dephosphorylation with prostate phosphomonoesterase, the mode of action of snake venom and spleen phosphodiesterases has been studied.

The snake venom (*Crotalus adamanteus*) diesterase preparation was obtained by acetone precipitation[3] followed by chromatography.[4] Incubation of pentathymidine tetraphosphate (T_5P_4) with this preparation gave the results shown in Table I. Thus, each of the lower homologs (T_4P_3, etc.) is formed successively and thymidine (T) appears last. The mononucleotide which accumulates is, as expected, thymidine 5′-phosphate. The data show that *degradation proceeds stepwise from the end bearing the 3′-hydroxyl group*. The degradation of oligonucleotides with 5′-phosphate end groups (T_nP_n), although much faster, also occurs stepwise. Further, in the hydrolysis of 3′-acetylated T_4P_4 the mononucleotide first released was 3′-acetyl-TP. This shows that the mode of degradation of these compounds is the same as found above and that hydrolysis does not begin from the end of the chain bearing the 5′-phosphate group.[5] Recent degradative experiments performed by Singer, Hilmoe and Heppel[6] on enzymatically synthesized polyribonucleotides confirm the present conclusions.

A phosphodiesterase from calf spleen has recently

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(3) J. F. Koerner and R. L. Sinsheimer, *J. Biol. Chem.*, **228**, 1049 (1957).

(4) H. G. Boman and U. Kaletta, *Biochim. Biophys. Acta*, **24**, 619 (1957).

(5) M. P. de Garilhe and M. Laskowski, *J. Biol. Chem.*, **223**, 661 (1956).

(6) Personal communication from Dr. Leon Heppel, National Institutes of Health, Bethesda.

been highly purified by Hilmoe.[7] The degradation of compounds of the type T_nP_{n-1} by this preparation was studied. It was found that, again, all the lower homologs were formed successively and that thymidine appeared only toward the end. However, the mononucleotide which accumulated was thymidine 3′-phosphate, as in the ribonucleotide series.[8] These results show that *the action of this enzyme as well is stepwise but begins from the end bearing the 5′-hydroxyl group.*

TABLE I

The reaction mixture consisted of M trihydroxymethylaminomethane, pH 8.9 (10 μl.), T_5P_4 (14 optical density units measured at 267 mμ), venom diesterase (31 μg. in 7 μl.) and water (20 μl.). Aliquots (6 μl.) were mixed with glacial acetic acid (1 μl.) and chromatographed in isopropyl alcohol–ammonia–water (7–1–2). The figures are the % of the total optical density in each aliquot.

Compound R_f	T_5P_4 0.05	T_4P_3 0.065	T_3P_2 0.24	T_2P 0.48	T 0.76	TP 0.18
Time, min.						
2	36	34	5	0	0	25
5	10	21	19	8	0	42
15	9	6	11	14	2	58
30	7	4	3	10	6	70
60	4	3	1	3	13	76

It should be noted that both the snake venom and spleen phosphodiesterase preparations hydrolyzed, even if slowly, cyclic oligonucleotides[1] in which the 3′-hydroxyl group is involved in an ester linkage with the 5′-phosphate group at the other end of the chain. It is therefore possible for these preparations to attack at a point within a polynucleotide chain (*cf.* ref. 3).

The stepwise and complementary action of the two phosphodiesterases is strikingly reminiscent of the two proteolytic enzymes, carboxypeptidase and (leucine) aminopeptidase, which degrade polypeptide chains from the opposite ends, and is promising for the structural and sequential analysis of polynucleotides.

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(7) R. J. Hilmoe, unpublished work. We are grateful for a gift of this preparation.

(8) L. A. Heppel and R. J. Hilmoe in "Methods in Enzymology," Vol. II, Academic Press Inc., New York, N. Y., 1955, p. 565.

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ENZYME STUDIES ON THE BIOSYNTHESIS OF VALINE IN YEAST

Sir:

In previous reports[1–3] based on isotope tracer data, a mechanism for the biosynthesis of valine was proposed, which involved α-acetolactic acid as an intermediate. This hypothesis has recently received support from Umbarger, *et al.*[4] In the present communication we wish to describe the properties of a cell-free preparation from *Saccharomyces cerevisiae* which converts acetolactic acid to α-ketoisovaleric acid, the keto analog of valine.

Fresh baker's yeast (Fleischmann) was disrupted in 0.02 M phosphate buffer, pH 7.2, by means of a high-speed refrigerated centrifuge shaker described by Shockman, *et al.*[5] The crude, cell-free supernatant obtained after centrifugation at 80,000 g was either used as such or was dialyzed against cold distilled water for 18–20 hours. The enzyme preparations were incubated at 37° with acetolactic acid, synthesized as described by Krampitz.[6] After 4 hours, the solution was deproteinized with tungstic acid, and the 2,4-dinitrophenylhydrazones of the keto acids were prepared, extracted and chromatographed on paper, essentially according to the procedure of Cavalini, *et al.*[7]

Results of a single, typical experiment, illustrative of the properties of this system, are shown in Table I. Mixtures of undialyzed extract and acetolactic acid produced a strong hydrazone spot, not observed in the absence of either enzyme or substrate. The identity of this material with the hydrazone of α-ketoisovaleric acid was established by identical R_f values in four different solvents, by identical absorption spectra over the wave length range of 350 to 700 mμ, and by reduction of material isolated from a strip chromatogram, according to Towers, *et al.*,[8] to a substance identical chromatographically with valine in three different solvent systems.

TABLE I

ENZYMATIC CONVERSION OF α-ACETOLACTATE TO α-KETOISOVALERATE

Each tube contained 80 μmoles potassium phosphate buffer, pH 8.0, and where indicated in the table, 0.05 mg. TPN, 0.5 mg. DPN, 1.0 mg. ATP, 27 μmoles neutralized acetolactate, pH 8.0, 27 μmoles K acetate, 27 μmoles ethanol, 15 μmoles magnesium sulfate, and 0.3 ml. of dialyzed or undialyzed enzyme, containing approximately 4 mg. protein (by ultraviolet absorption) and representing 0.15 g. of fresh yeast. Total volume was 1.6 ml. and incubation was conducted 4 hours in air at 37°.

Enzyme preparation	Acetolactate	Cofactor(s)	α-Ketoisovalerate micromoles
Crude	+	—	1.28
Crude	+	TPN	2.10
Dialyzed	+	—	0.06
Dialyzed	+	Crude heated	1.83
Dialyzed	+	DPN	0.14
Dialyzed	+	DPN, ATP, Mg^{++}	2.19
Dialyzed	+	TPN	2.97
Dialyzed[a]	Acetate + ethanol	TPN	0.02

[a] This was run to check the effects of acetate and ethano present as a result of hydrolysis of the ethyl acetoxyacetolactate.

The values given in Table I were obtained by elution of the α-ketoisovaleric acid hydrazone spots

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