creases with pH and concentration of O_2^* and is inhibited by hydrogen peroxide. The yield appears to be independent of dosage rate in the range from 2.19×10^{20} to 0.464×10^{20} ev./l. min. The initial yields of normal oxygen from the enriched oxygen appear in Table I.

TABLE I

γ-RAY INITIATED $O^{16}O^{18}$–H_2O^{16} EXCHANGE IN ALKALINE SOLUTION

Expt.	pH	$(O_2^*)_0$, mM	Dosage rate (ev./l. min.)	$(H_2O_2)_0$, mM	G_{O_2}[a]	G_{O_2}/mM O_2^*
1	2.15	1.17	2.19×10^{20}	0.0	0.75	0.64
2	6.0	1.21	2.19×10^{20}	.0	0.90	0.74
3	8.98	1.17	2.19×10^{20}	.0	2.4	2.1
4	9.65	0.68	2.19×10^{20}	.0	5.7	8.4
5	11.32	1.20	2.19×10^{20}	.0	36	30
6	11.91	0.987	2.19×10^{20}	.0	58	59
7	11.89	0.796	0.464×10^{20}	.0	48	60
8	11.65	0.114	2.19×10^{20}	.0	9.4	82
9	12.65	1.08	2.19×10^{20}	.0	120	111
10	11.62	1.18	2.19×10^{20}	.156	10.3	8.7

[a] G_{O_2} = molecules O_2 formed/100 ev. of absorbed γ-ray energy. (Energy absorption is based on $G_{Fe^{+++}} = 15.5$ for ferrous sulfate dosimeter.)

The yield of normal oxygen rises sharply in alkaline solution in the region above pH 9. Since the yield of free radicals produced by Co^{60} γ-rays is 2.61 H and OH/100 ev.,[1] it is evident that under the conditions studied, as many as 40 O_2^* molecules are converted to normal O_2 molecules/radical pair formed in the solution. This chain reaction is terminated by reaction with hydrogen peroxide as can be seen by comparing experiments 5 and 6 with 10.

During the course of this investigation it was also established that there is no thermal exchange of dissolved O_2^* with OH^- in aqueous solutions of pH 11.8. However, it was found that a thermal exchange of 0.5×10^{-6} M O_2^*/min. occurred in a solution containing 1.15 mM O_2^* and 0.18 mM normal hydrogen peroxide at a pH of 11.75. This rate is very small compared to the gamma ray induced rate in the experiments carried out at a dosage rate of 2.19×10^{20} ev./l. min. (see experiment 10). Owing to the fact that only of the order of 10^{-5} M hydrogen peroxide is formed during the course of the irradiation, a correction for the contribution of the thermal rate would be difficult to estimate without a more complete knowledge of the effect of hydrogen peroxide concentration on the exchange reaction.

As a result of recent work[2] on the free radical induced deuterium-water reaction, we have suggested the equilibrium

$$OH = O^- + H^+$$

to explain the drop in yield of hydrogen deuteride at a pH of 9.0. Since the $O_2^* + H_2O = O_2 + H_2O^*$ chain reaction begins at this pH and continues to develop at a pH of 12.65 we postulate participation of O^-, O_2^* and OH^- as indicated in the following mechanism

$$\left.\begin{aligned} H_2O + \gamma\text{-rays} &= H + OH \\ OH &= O^- + H^+ \end{aligned}\right\} \text{Initiation}$$

$$\left.\begin{aligned} O^- + O_2^* &= O^{*-} + O_2 \\ O^{*-} + OH^- &= O^- + O^*H^- \end{aligned}\right\} \text{Propagation}$$

$$\left.\begin{aligned} H + O_2^* &= HO_2^* \\ HO_2^* &= H^+ + O_2^{*-} \\ O^{*-} \text{ or } O^- + HO_2^- &= O^*H^- \text{ or } OH^- + O_2^- \\ O^{*-} \text{ or } O^- + O_2^{*-} &= O^{*-} \text{ or } O^- + O_2^* \end{aligned}\right\} \text{Termination}$$

The relatively stable O_2^- molecule ion and hydrogen peroxide or the ion HO_2^- are suggested as chain terminators.

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VITAMIN B_{12}. XXI. CRYSTALLINE α-RIBAZOLE PHOSPHATE AND ITS SYNTHESIS

Sir:

A crystalline phosphate of α-ribazole (1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole) has been obtained both as a degradation product of vitamin B_{12} and by synthesis. An amorphous barium salt of this phosphate(s) obtained by degradation of vitamin B_{12} was reported previously.[1]

α-Ribazole (2′ or 3′)-phosphate was separated from the acid hydrolyzate of vitamin B_{12} as the lead salt. The lead salt was converted to the phosphate with hydrogen sulfide; after countercurrent distribution (*n*-butanol–water) of the crude product, it crystallized from water–acetone mixtures. The crystalline phosphate melted at 240–241° dec. (micro-block). *Anal.* Calcd. for $C_{14}H_{19}N_2O_7P$: C, 46.93; H, 5.34; N, 7.82; P, 8.65. Found: C, 46.88; H, 5.57; N, 7.54; P, 8.39. The absorption spectra of aqueous solutions were: at *ca.* pH 2, maxima at 277 mμ ($E^{1\%}_{1\text{ cm.}}$ 217) and 285 mμ ($E^{1\%}_{1\text{ cm.}}$ 202); and at *ca.* pH 11, maxima at 249 mμ ($E^{1\%}_{1\text{ cm.}}$ 191), 280 mμ ($E^{1\%}_{1\text{ cm.}}$ 144), and 288 mμ ($E^{1\%}_{1\text{ cm.}}$ 136).

A crystalline brucine salt of α-ribazole (2′ or 3′)-phosphate also was obtained from the acid hydrolyzate of vitamin B_{12}. A methanol solution of the phosphate obtained from the above-mentioned lead salt was treated with a methanol solution of brucine. Concentration of the solution and cooling gave the crystalline dibrucine salt. It also crystallized from water; m.p. 169–175° (micro-block).

Anal. Calcd. for $C_{60}H_{71}N_6O_{15}P$: C, 62.81; H, 6.24; N, 7.33; P, 2.71. Found: C, 62.82; H, 6.28; N, 7.39; P, 2.85.

α-Ribazole (2′ or 3′)-phosphate was best prepared synthetically by phosphorylation of 5′-trityl-α-ribazole with diphenylchlorophosphonate.[2] After removal of the trityl and phenyl groups by acid hydrolysis, α-ribazole phosphate was isolated as the lead salt, which was decomposed with hydro-

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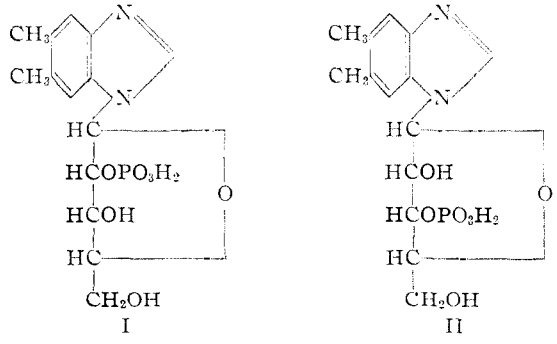
gen sulfide. The product crystallized from water–acetone. After one recrystallization, α-ribazole phosphate melted at 235–236° dec. (capillary) 240–241° (micro-block). The melting point of this material in admixture with the vitamin B_{12} degradation product was not depressed. *Anal.* Found: C, 46.72; H, 5.25; N, 7.81; P, 8.33. The absorption spectra of aqueous solutions were: at *ca.* *p*H 2, maxima at 277 mμ and 285 mμ; at *ca.* *p*H 11, maxima at 249 mμ, 280 mμ, and 288 mμ.

The phosphorylation of 5′-trityl-α-ribazole was also effected by means of dibenzylchlorophosphonate.[3] The benzyl groups were subsequently removed by hydrogenolysis. The product was purified as the crystalline dibrucine salt, m.p. 169–173° (capillary). *Anal.* Found: C, 62.66; H, 6.34; N, 7.31; P, 2.46.

Brown and Todd[4] have separated adenylic acids a and b by paper chromatography using a solvent system (5% aqueous disodium hydrogen phosphate–isoamyl alcohol) developed by Carter.[5] Paper strip chromatography of the crystalline phosphate from vitamin B_{12} and the synthetic α-ribazole phosphate with this system showed that the two samples were identical and consisted of only one isomer (2′ or 3′ phosphate), having an R_F value of 0.74. The α-ribazole phosphate was detected as a fluorescent spot after the dried paper chromatogram had been sprayed with 2% acetic acid. Furthermore, when the two samples of α-ribazole phosphate were treated by the method of Brown and Todd[4] for the isomerization of the adenylic acids, *i.e.*, by heating under reflux in 80% acetic acid for ten minutes, each was converted into a mixture of approximately equal parts of the 2′- and 3′-isomers. The isomers had R_F values of 0.78 and 0.74.

The identification of this crystalline phosphate as the 2′-phosphate (I) or the 3′-phosphate (II) is



I II

not possible on this evidence, and this differentiation is comparable to the situation on adenylic acids a and b.[4] Furthermore, the possibility of phosphoryl migration during the acid hydrolysis of vitamin B_{12} indicates that the position of the linkage of the phosphate group to ribose in this crystalline α-ribazole phosphate is not necessarily the same as it is in vitamin B_{12}.

Dr. Gladys A. Emerson of the Merck Institute for Therapeutic Research tested α-ribazole phosphate for vitamin B_{12} activity[6] in rats and found that it has substantially the same activity as α-ribazole, or about one four-hundredth the activity of vitamin B_{12}.

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ON THE INTERNAL ROTATION OF A POLYPEPTIDE CHAIN

Sir:

A recent publication[1] states that an 11-atom ring structure of a polypeptide chain is possible, consistent with the published X-ray data, if the azimuthal angle of internal rotation about –NH–CO– axis is different by about 30° from that of the planar peptide structure. However, Pauling and Corey consider that such a structure is unacceptable.[2] During the past years we have carried out some experimental work to determine the internal rotation about various single bonds contained in a polypeptide chain. For example, in the case of CH_3–CO–NH–CH_3 it was proved by the ultraviolet measurement that this molecule has a planar configuration and the deviation of 30° from the planar position seems improbable.[3] According to our infrared, Raman and dipole measurements on this substance, the two CH_2– groups are in the *trans* position with respect to each other in the liquid state and in aqueous and carbon tetrachloride solutions of various concentrations.[3] We can derive the same conclusion for the structure of the peptide bonds of a polypeptide chain from the infrared measurement. Therefore, we cannot agree with those Pauling and Corey models which have the *cis* configurations of peptide bonds.[4]

Pauling and Corey also discussed the internal rotation about single bonds of a polypeptide chain other than that about peptide bonds.[5] As to the internal rotation we have been publishing many papers[6] and our polypeptide model is based on the conclusion of these papers.[7] The presence of six potential minima in one rotation about a single bond suggested by Pauling and Corey[5] is not compatible with our experimental result. Generally we have three potential minima in one complete internal rotation.[6] We are planning to publish further our experimental results concerning the

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