

**The pH Dependence of the Rate Catalyzed by Trypsin.**—On a logarithmic scale the pH dependence of the trypsin-catalyzed reaction is extremely slight from pH 6 to 10, quite in contrast to the behavior of the other endopeptidases. However, on a linear scale a pH dependence much larger than the experimental uncertainty becomes evident (Fig. 1b). Studies on benzoyl-L-arginine ethyl ester<sup>20</sup> indicate a  $pK_a$  for the enzyme-substrate complex of 6.25 and no  $pK_b$  was evident up to pH 10. Various values for  $pK_a$  were assumed and the pH dependence predicted. As can be seen in Fig. 1b, the observed pH dependence can be predicted below pH 9 assuming  $pK_a = 6.6$ . Below pH 9, then, the pH dependence of the rate is controlled by the

(20) H. Gutfreund, *Trans. Faraday Soc.*, **51**, 441 (1955).

charge state of the enzyme and is independent of the charge state of the substrate. A variety of models involving the charge state of the enzyme and the charge state and conformation of the polylysine were considered in an attempt to explain the pH behavior above pH 9. Several of these predicted a decrease in rate that was qualitatively correct. None, however, stood out over the others. However, the data must be extended to higher pH and the Michaelis constants determined over the entire pH range before a meaningful interpretation can be given.

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## On the Thermochemistry of Alkyl Polyoxides and Their Radicals<sup>1</sup>

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From data on heats of formation of oxides and peroxides, a new value for the partial molar heat of formation of the group  $O-(O)_2$  is selected. Its value is  $\Delta H_f^\circ(O-(O)_2) = +19 \pm 4$  kcal./mole. Using this and other more accurate data, it is possible to deduce  $\Delta H_f^\circ$  for polyoxides  $RO_nR'$  with  $n = 2, 3, 4$ , etc., and  $R$  and  $R' = H$ , alkyl, etc. Together with data on  $\Delta H_f^\circ$  for radicals, it is shown that it is unlikely that tetroxides can be produced or isolated above 80 to 100°K. The trioxides on the other hand appear to have reasonable thermal stability and recent observations by Czapski and Bielski<sup>2a</sup> on  $H_2O_3$  production are in accord with these expectations. Free-radical methods will not produce very large amounts of these species. In the case of  $R_2O_3$ , where  $R$  is a tertiary radical, this is not the case and it is proposed that the recent production of a polyoxide by Milas and Djokic<sup>2b</sup> is very likely  $t\text{-BuO}_3\text{-}t\text{-Bu}$  rather than the claimed tetroxide.

### Introduction

In a recent paper<sup>3</sup> it was estimated that the species  $H_2O_4$  is thermodynamically unstable above 80°K. with respect to dissociation into  $2HO_2$  radicals. The lower homolog,  $H_2O_3$ , was estimated to be sufficiently stable to be isolable at 200°K. Despite this, it was shown on kinetic grounds that attempts which had been made to produce it from gas-phase or gas-solid free-radical reactions were unlikely to succeed. Since then data have appeared<sup>4</sup> which further substantiated the kinetic conclusions on the unlikelihood of  $H_2O_3$  or  $H_2O_4$  preparation. However, some recent results on aqueous oxidation-reduction systems suggest the formation of  $H_2O_3$  and  $HO_3^-$  via alternate routes. In addition some experiments<sup>2b</sup> on the alkyl derivatives of peroxides have given direct support to the reality of the compounds  $R_2O_3$  or  $R_2O_4$ . In the light of these findings it was felt worthwhile reviewing the thermochemical data on the hydrogen polyoxides and extending the methods of analysis to the alkyl compounds, their radicals, and derivatives.

**Thermodynamic Estimates.**—The basis for the method of estimation of the heats of formation of the polyoxides derives from the principle of additivity of group properties as described by Benson and Buss<sup>5</sup> in

(1) This development was partly supported at Stanford Research Institute by a general project on "Reaction of Organic Compounds with Oxygen."

(2) (a) G. Czapski and B. H. J. Bielski, *J. Phys. Chem.*, **67**, 2180 (1963); (b) N. A. Milas and S. M. Djokic, *Chem. Ind. (London)*, 405 (1962).

(3) S. W. Benson, *J. Chem. Phys.*, **33**, 306 (1960).

(4) P. A. Giguère, *ICSU Rev.*, **4**, 172 (1962).

(5) S. W. Benson and J. H. Buss, *J. Chem. Phys.*, **29**, 546 (1958). Follow-

conjunction with the less accurate principle of bond additivities. The former was found to give values of  $\Delta H_f^\circ$  to within  $\pm 1$  kcal. in about 1000 cases, and the polyoxides should fit the scheme quite well. The problem is one of establishing the partial group value of  $\Delta H_f^\circ$  for the group  $O-(O)_2$ , i.e., an oxygen atom bonded to two neighboring O atoms. In the preceding paper,<sup>3</sup> this value was estimated from bond energies. Such estimates can be made from the values of  $\Delta H_f^\circ(H_2O)$  and  $\Delta H_f^\circ(H_2O_2)$  which would yield the value  $\Delta H_f^\circ(O-(O)_2) = +25$  kcal. with a maximum expected error of  $\pm 6$  kcal. based on a number of similar cases. If, however, the estimates are made from the data on  $\Delta H_f^\circ(CH_3OCH_3)$  and  $\Delta H_f^\circ(CH_3OOCH_3)$ ,<sup>6</sup> then we estimate  $\Delta H_f^\circ(O-(O)_2) = +13$  kcal. with similar error limits. The mean value of  $\Delta H_f^\circ(O-(O)_2) = +19 \pm 4$  kcal. seems a reasonable compromise of both these estimates and is to be preferred on purely empirical grounds.

This new group value of  $\Delta H_f^\circ[O-(O)_2]$  then yields, together with the other appropriate group values,<sup>5</sup> the estimated heats of formation shown in Table I. To calculate bond dissociation energies for the compounds listed we use the  $\Delta H_f^\circ$  for values the radicals shown in Table II.

**Polyoxide Stability. Tetroxides.**—The values listed for  $\Delta H_f^\circ$  of  $H_2O_3$  and  $H_2O_4$  are lower by about 2 and 4 kcal., respectively, than the ones suggested earlier, but using the nomenclature adopted in this article  $\Delta H_f^\circ(O-(O)_2)$  represents the partial molar group contribution to the total heat of formation of a molecule containing the group  $O-(O)_2$ , an oxygen atom joined to two neighboring O atoms. As an example,  $H_2O_2$  contains only two identical groups  $O-(H)(O)$ , while  $H_2O_3$  contains the same two  $O-(H)(O)$  groups plus the  $O-(O)_2$  group.

(6) See compilation in S. W. Benson, *ibid.*, **40**, 1007 (1964).

TABLE I  
ESTIMATED HEATS OF FORMATION OF SOME POLYOXIDES  
IN THE GAS PHASE

Compound	$\Delta H_f^\circ$ , kcal./mole <sup>a</sup>
H <sub>2</sub> O <sub>2</sub>	-32.5
H <sub>2</sub> O <sub>3</sub> <sup>b</sup>	-13.5 ± 4
H <sub>2</sub> O <sub>4</sub> <sup>b</sup>	+ 5.5 ± 8
CH <sub>3</sub> O <sub>2</sub> CH <sub>3</sub>	-32
CH <sub>3</sub> O <sub>3</sub> CH <sub>3</sub> <sup>b</sup>	-13 ± 4
CH <sub>3</sub> O <sub>4</sub> CH <sub>3</sub> <sup>b</sup>	+ 6 ± 8
CH <sub>3</sub> O <sub>2</sub> H	-32
CH <sub>3</sub> O <sub>3</sub> H <sup>b</sup>	-13 ± 4
CH <sub>3</sub> O <sub>4</sub> H <sup>b</sup>	+ 6 ± 8
<i>t</i> -BuO <sub>2</sub> - <i>t</i> -Bu	-85
<i>t</i> -BuO <sub>3</sub> - <i>t</i> -Bu <sup>b</sup>	-66 ± 4
<i>t</i> -BuO <sub>4</sub> - <i>t</i> -Bu <sup>b</sup>	-47 ± 8
<i>t</i> -BuO <sub>2</sub> H	-59
<i>t</i> -BuO <sub>3</sub> H <sup>b</sup>	-40 ± 4
<i>t</i> -BuO <sub>4</sub> H <sup>b</sup>	-21 ± 8

<sup>a</sup> Values are taken from ref. 6 except where error limits are given. <sup>b</sup> Estimates made by the present method.

TABLE II  
 $\Delta H_f^\circ$  FOR SOME OXYGEN-CONTAINING RADICALS

Radical	$\Delta H_f^\circ$ , kcal./mole
O	59.6 <sup>a</sup>
HO	9.0 ± 0.3 <sup>b</sup>
HO <sub>2</sub>	5.0 ± 2 <sup>c</sup>
HO <sub>3</sub>	24.0 ± 6 <sup>d</sup>
CH <sub>3</sub> O	2 ± 2 <sup>e</sup>
CH <sub>3</sub> O <sub>2</sub>	5.5 ± 3 <sup>f</sup>
CH <sub>3</sub> O <sub>3</sub>	24.5 ± 6 <sup>d</sup>
<i>t</i> -BuO	-24 ± 1 <sup>g</sup>
<i>t</i> -BuO <sub>2</sub>	-21.5 ± 2.5 <sup>f</sup>
<i>t</i> -BuO <sub>3</sub>	- 2.5 ± 6 <sup>d</sup>

<sup>a</sup> JANAF Tables, 1962. <sup>b</sup> P. Gray, *Trans. Faraday Soc.*, **55**, 408 (1959). <sup>c</sup> Ref. 7. <sup>d</sup> From preceding member and  $\Delta H_f^\circ(\text{O}-(\text{O})_2)$ . <sup>e</sup> From pyrolysis of CH<sub>3</sub>OCH<sub>3</sub>, S. W. Benson and K. H. Anderson [*J. Chem. Phys.*, **39**, 1677 (1963)] give an estimate of  $D(\text{CH}_3\text{O}-\text{H}) = 102$  kcal. and self-consistency of  $E_{\text{act}}$  for dissociation of peroxides. <sup>f</sup> Assumption that  $D(\text{RO}_2-\text{H}) = D(\text{HO}_2-\text{H}) = 89.5$  kcal.; see ref. 7. <sup>g</sup>  $E_{\text{act}}$  for pyrolysis of dtBP: L. Batt and S. W. Benson, *J. Chem. Phys.*, **36**, 895 (1962).

within the error limits previously cited. If we select a new value<sup>7</sup> of  $\Delta H_f^\circ(\text{HO}_2) = +5.0 \pm 2$  kcal./mole, which implies the bond dissociation energies  $D(\text{HO}_2-\text{H}) = 89.5$  and  $D(\text{H}-\text{O}_2) = 47.0$  kcal., we find that  $D(\text{HO}_2-\text{OH}) = 27.5 \pm 6$  and  $D(\text{HO}_2-\text{O}_2\text{H}) = 4.5 \pm 12$  kcal. These estimated stabilities of H<sub>2</sub>O<sub>3</sub> and H<sub>2</sub>O<sub>4</sub> are about 6 kcal. greater than our earlier estimates. H<sub>2</sub>O<sub>3</sub> is now expected to be stable below 25°, but H<sub>2</sub>O<sub>4</sub> is not expected to be stable at temperatures much above 100°K. However, the uncertainty in bond energy is now so large as to make any very precise estimates of its region of stability of dubious value.

Using the value of  $\Delta H_f^\circ(\text{CH}_3\text{O}) = +2$  kcal./mole,<sup>6</sup> we find  $\Delta H_f^\circ(\text{CH}_3\text{O}_2) = 5.5 \pm 2$  kcal./mole,  $D(\text{CH}_3\text{O}_2-\text{OCH}_3) = 20.5 \pm 6$ ,  $D(\text{CH}_3\text{O}_2-\text{O}_2\text{CH}_3) = 5 \pm 12$ ,  $D(\text{CH}_3\text{O}_2-\text{OH}) = 26.5 \pm 6$ , and  $D(\text{CH}_3\text{O}-\text{O}_2\text{H}) = 20.5 \pm 6$  kcal. with similar values to be expected for the *t*-Bu oxides<sup>8</sup> or for alkyl oxides in general, since the RO-OR bond energies seem to be very close to each other.<sup>6</sup> We see that the thermodynamic stability of the alkyl and hydrogen polyoxides are very similar; that is, the

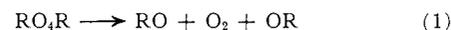
(7) S. N. Foner and R. L. Hudson, *J. Chem. Phys.*, **36**, 2681 (1962).

(8) For the *t*-Bu series, using the corrected value for  $\Delta H_f^\circ(\text{t-BuO}) = -24.0$  kcal./mole,  $\Delta H_f^\circ(\text{t-BuO}_2) = -21.5 + 2$  kcal./mole and  $D(\text{t-BuO}_2-\text{O}-\text{t-Bu}) = 20.5 \pm 6$ ,  $D(\text{t-BuO}_2-\text{O}_2-\text{t-Bu}) = 4 \pm 12$ ,  $D(\text{t-BuO}_2-\text{OH}) = 27.5 \pm 6$ ,  $D(\text{t-BuO}-\text{O}_2\text{H}) = 21 \pm 6$ , and  $D(\text{t-BuO}_2-\text{O}_2\text{H}) = 4.5 \pm 12$  kcal.

compounds RO<sub>3</sub>R and RO<sub>3</sub>H are expected to be stable below 250°K., while RO<sub>4</sub>R and RO<sub>4</sub>H would be stable below 100°K.

It should be emphasized that we use the term stability here in a kinetic sense to indicate that the dissociation into radicals would have a half-life in excess of 1 hr., assuming that the first-order rate constant for the dissociation has an Arrhenius *A* factor of 10<sup>15</sup> sec.<sup>-1</sup>.<sup>9</sup> This criterion further assumes that once formed in a dissociation step, the radicals would then react to produce final products by very rapid disproportionation steps. We omit from consideration for the moment the very real prospect that they would set up chains by attack on the parent compounds. This would lower even further the temperature for kinetic stability.

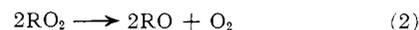
While the alkyl tetroxides are not very stable towards dissociation into two peroxy radicals, their chief mode of decomposition would appear to be the concerted, exothermic reaction to produce two alkoxy radicals and O<sub>2</sub>.



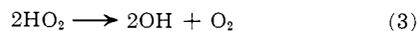
This reaction is about 2 ± 10 kcal. exothermic and almost independent of the group R. Even if we take the extreme limits of uncertainty and consider the reaction endothermic by 8 kcal., it would yield a stability temperature of 100°K. This makes somewhat dubious the claims of Milas and Djokic<sup>2b</sup> to have isolated the tetraoxide of *t*-Bu radicals at temperatures below 240°K.

The compound RO<sub>4</sub>H has  $D(\text{RO}_2-\text{O}_2\text{H}) = 4.5 \pm 13$  kcal. which would give it the same stability temperature as RO<sub>4</sub>R. The dissociation into RO + OH + O<sub>2</sub> is, however, endothermic by 5 ± 10 kcal. and so again represents a lower energy path for decomposition than the peroxy radical formation. This limits the existence of RO<sub>4</sub>H to temperatures below 60 or 180°K. if we accept the largest value for the expected error in estimated endothermicity.

In contrast to RO<sub>4</sub>R, HO<sub>4</sub>H is stable with respect to the formation of 2OH + O<sub>2</sub>, the endothermicity of the reaction being 12.5 ± 8 kcal. This is therefore a less favorable dissociation than the direct split into 2HO<sub>2</sub> radicals. This also helps explain the observation that the gas phase pyrolysis of H<sub>2</sub>O<sub>2</sub> is not a chain reaction,<sup>10,11</sup> while RO<sub>2</sub>H does undergo chain reaction by the relatively fast, nonterminating, exothermic step<sup>6</sup>



The similar reaction



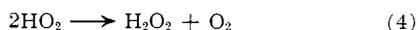
is endothermic by 8 ± 4 kcal. and thus is expected to

(9) We are extending to polyoxides the empirical result that radical-radical reactions in the gas phase and in non-H-bonding solvents appear to have zero activation energy. On this basis the activation energies for dissociation may be taken to be equal to the bond dissociation energies. In the same way, we are also assuming that the high frequency factors (10<sup>15</sup> to 10<sup>16</sup> sec.<sup>-1</sup>) characteristic of first-order peroxide decompositions are also applicable to the hydrogen polyoxides. A lower limit of 10<sup>14.3</sup> sec.<sup>-1</sup> for such *A* factors is indicated from very conservative transition-state models. An error of ±1 power of 10 would introduce a corresponding error of ±5% in the absolute values of the ceiling temperatures.

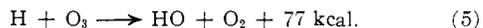
(10) P. A. Giguère and I. D. Liu, *Can. J. Chem.*, **35**, 283 (1957); W. Forst, *ibid.*, **36**, 1308 (1958); R. R. Baldwin and D. Bratten, Eighth International Symposium on Combustion, Pasadena, Calif., 1960, Williams and Wilkins Co., Baltimore, Md., 1962, p. 110.

(11) D. E. Hoare, J. B. Protheroe, and A. D. Walsh, *Trans. Faraday Soc.*, **55**, 548 (1959).

be of negligible importance relative to the rapid disproportionation reaction<sup>12,13</sup>



**Polyoxide Stability. Trioxides.**—The bond dissociation energies for the trioxides, for their lowest energy paths, are all similar and all close to  $20 \pm 6$  kcal. In accordance with our earlier conclusions,<sup>3</sup> this would suggest the trioxides as the most reasonable candidates for low temperature isolability. The principal problem would be the method of preparation. In our previous discussion of  $\text{H}_2\text{O}_3$ ,<sup>3</sup> it was pointed out that the addition of H atoms to  $\text{O}_3$  was a very unlikely source because of the great exothermicity of the reaction



It is very unlikely that any  $\text{HO}_3$  will survive the addition of H to  $\text{O}_3$ . Even if a small amount of excited  $\text{HO}_3$  were thermalized in a solid matrix, we see (Table II) that it is unstable with respect to the dissociation into  $\text{HO} + \text{O}_2$  by  $15 \pm 6$  kcal. and again unlikely to survive for participation in any further radical reactions. These conclusions are very much in accord with the experiences of Giguère and Chin,<sup>14</sup> who found that cold, gaseous  $\text{O}_3$  exploded when exposed to H atoms, while solid films gave only  $\text{H}_2\text{O}_2 + \text{H}_2\text{O}$ .

The alternative radical route to  $\text{H}_2\text{O}_3$  from the cross-addition of  $\text{HO} + \text{HO}_2$  can be shown to be very unlikely relative to the completing reactions of disproportionation which are known to be very rapid.<sup>13</sup> However, in the experiments of Czapski and Bielski,<sup>2a</sup> these arguments must be moderated since they claimed to observe only very small amounts of  $\text{H}_2\text{O}_3$ , which would in fact be in the range to be expected from the steady-state calculations. They generated  $\text{HO}_2$  in a flow system by exposing  $\text{O}_2$ -saturated  $\text{H}_2\text{O}$  to an intense electron beam. Titrating downstream at various times after irradiation with  $\text{Fe}^{+2}$ , they observed an oxidizing intermediate

(12) J. H. Knox and C. H. J. Wells, *Trans. Faraday Soc.*, **59**, 2786 (1963), have suggested that (3) can still be important in the gas-phase oxidation of hydrocarbons at  $360^\circ$  if it occurs *via* a brief formation of excited  $\text{H}_2\text{O}_4$  which then goes on to decompose *via* reaction 3 with a large frequency factor. Their conclusion is, however, based on false reasoning since the rate-limiting process is the rate of formation of  $\text{H}_2\text{O}_4$  with the necessary 5 kcal. of activation energy. It does not matter how rapidly the excited  $\text{H}_2\text{O}_4$  then decomposes. Using values of  $10^{10}$  l./mole-sec. for the A factors for  $\text{H}_2\text{O}_4$  formation and reaction 4, the latter will be favored by about 100-fold at  $650^\circ\text{K}$ . because of the energy requirement for (3). We are again assuming that radical-radical reactions in the gas phase have zero activation energy.<sup>9</sup> This is in apparent "not real" contradiction to the observation (ref. 13) that in 0.8 N  $\text{H}_2\text{SO}_4$  the disproportionation of  $2\text{HO}_2$  has about a 6-kcal. activation energy. In H-bonded liquids, the strength of H bonding is sufficiently great so that any change in the relative number of H bonds going from solvated species to transition state could contribute something of this order of magnitude to the activation energy. In the gas state or in nonpolar solvents, one would expect such activation energies to vanish.

(13) B. H. J. Bielski and E. Saito, *J. Phys. Chem.*, **66**, 2266 (1962), reported a rate constant  $2.4 \times 10^6$  l./mole-sec. from e.s.r. techniques and confirmed this (ref. 2a) using a titration technique.

(14) P. A. Giguère and D. Chin, *J. Chem. Phys.*, **31**, 1685 (1959).

which decayed by a first-order rate law (at all pH values) to a nonoxidizing species. Its half-life was a maximum of  $\sim 2$  sec. at pH 1 to 2 and some 25-fold smaller at pH 0 or pH 3. The maximum observed lifetime is compatible with any of the expected limits on  $D(\text{HO}_2\text{-OH})$ . The lower limit of 21.5 kcal. would give a lifetime at  $25^\circ$  of about 10 sec. In fact, their maximum observed lifetime of 2 sec. puts a lower limit on  $D(\text{HO}_2\text{-OH})$  in solution of about 20 kcal. if indeed this is their species.

This estimate is quite independent of the observation that the "observed"  $\text{H}_2\text{O}_3$  disappears by an acid-catalyzed mechanism. It simply means that any homolytic process is slower than this observed catalyzed decomposition.

In the case of the alkyl trioxides, the energetics of radical addition to  $\text{O}_3$  are equally unfavorable.  $\text{RO}_3$  is unstable with respect to cleavage into  $\text{RO} + \text{O}_2$  by  $22 \pm 8$  kcal. and thus a very unlikely intermediate. The radical addition of  $\text{RO}$  to  $\text{RO}_2$  is, however, more favorable than in the case of  $\text{HO} + \text{HO}_2$  since there is only one competing disproportionation for primary or secondary radicals.



For tertiary radicals,  $\text{R}'_3\text{CO}$  and  $\text{R}_3\text{CO}_2$ , however, no fast disproportionation is possible and  $\text{R}_2\text{O}_3$  formation should be favored. From this point of view it seems most likely that the white solid isolated by Milas and Djokic<sup>2b</sup> was more likely  $t\text{-Bu}_2\text{O}_3$  than the  $t\text{-Bu}_2\text{O}_4$  which they reported. Their identification was somewhat tenuous in that they reported no data on analysis or stoichiometry, only a descriptive statement concerning the evolution of  $\text{O}_2$  and  $t\text{-Bu}_2\text{O}_2$  from the solid. However, from their method of production, which involved the formation of large initial yields of  $t\text{-Bu}_2\text{O}_2$  and  $\text{O}_2$ , it is clear that they must have had large amounts of  $t\text{-BuO}$  radicals as well as  $t\text{-BuO}_2$ , and it is very unlikely that with  $t\text{-Bu}_2\text{O}_2$  as the major product that the favored  $t\text{-Bu}_2\text{O}_3$  will not be formed in considerable excess of any  $t\text{-Bu}_2\text{O}_4$ .

One final comment seems appropriate in reference to the polyoxides, and this concerns their use as rocket fuels. It can be seen from Table I that  $\text{H}_2\text{O}_3$  is not as energetic an oxidizer as  $\text{O}_3$  or  $\text{O}_2$ . In a similar vein, the more stable  $\text{CH}_3\text{O}_3\text{CH}_3$  is a less energetic oxidizer than  $\text{O}_3$  or  $\text{O}_2$  and only slightly more energetic than appropriate  $\text{O}_2 + \text{C}_2\text{H}_6$  mixtures. Even in the radical series (Table II) we note that  $\text{HO}_3$  is a less energetic oxidizer than  $\text{O}_3$ , while  $\text{HO}_2$  is only slightly more energetic than  $\text{O}_2$ .

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