was poured into water and extracted with chloroform. On evaporation, a residue (370 mg.) was left which was chromatographed through activated alumina (20 g.). The following elutions were made: chloroform–benzene 3:2 (50 mg.) and 9:1 (30 mg.), pure chloroform (traces) and chloroform with 1% methanol (60 mg.) and with 5% methanol (100 mg.). All these fractions were amorphous and could not be crystallized. Infrared spectra showed that only in the first fraction the carbonyl absorption had completely disappeared. In all others, weak absorptions at 1697 cm.$^{-1}$ were present and they were probably mixtures.

Elatericinic Acid.—Elatericin B (5 g.) in ethanol (125 ml.) was added to an 8% solution (125 ml.) of sodium hydroxide and heated to reflux under nitrogen for seven hours. Part of the ethanol was evaporated under reduced pressure and diluted with water (50 ml.). The mixture was then acidified with hydrochloric acid and extracted several times with ether. The elatericinic acid formed was extracted with a solution of sodium bicarbonate 5% and after acidification of the solution extracted with ether. Evaporation of the solvent left a residue (2.5 g.) which crystallized from toluene–petroleum ether by freezing, m.p. 140–143° dec. (sinters at 120°).

Anal. Calcd. for $C_{28}H_{44}O_8$: C, 66.11; H, 8.72; mol. wt., 508.6. Found: C, 66.45; H, 8.51, equiv. wt., 505, determined by titration in dry pyridine with tetrabutylammonium hydroxide in benzene–methanol (9:1) using thymol blue as indicator.[21]

(21) R. H. Cundiff and P. C. Markunas, *Anal. Chem.*, **28**, 792 (1956).

The methyl ester of elatericinic acid was prepared with a solution of diazomethane in ether. When the gas evolution subsided, the solvent was evaporated. The residue was amorphous and did not crystallize.

Reduction of Methyl Elatericinate to Elatericinol.—A solution of methyl elatericinate (1.8 g.) in dry ether (100 ml.) was added slowly to a stirred solution of lithium aluminum hydride (3.5 g.) in ether (100 ml.). The mixture was heated for 60 hours and hydrolyzed with dilute acid. The ether layer was washed with water, dried and evaporated leaving a residue (750 mg.). The aqueous layer and the washings were combined and continuously extracted with chloroform for 24 hours yielding on evaporation of the solvent an amorphous substance (450 mg.); microcrystals from xylene, m.p. 127–130° (hot-stage). Both substances were found to be identical by their infrared spectra; ν_{max} 3450 cm.$^{-1}$ (s).

Periodic Acid Oxidation of Elatericinol–Norelatericinone.—Periodic acid (200 mg.) in 3 ml. of water was added to a solution of elatericinol (80 mg.) in ethanol (5 ml.) and left overnight at room temperature. The mixture was then diluted with water and extracted with chloroform. The chloroform layer was washed and distilled, leaving an amorphous substance (56 mg.); ν_{max} 1733 (cyclopentanone) and 1698 cm.$^{-1}$.

Cyclohexanedione was prepared from cyclohexanone and selenious acid in dioxane water according to reference 22: b.p. 78–80° (18 mm.), m.p. 38°, yield 55%.

(22) *Org. Syntheses*, **32**, 35 (1952).

REHOVOTH, ISRAEL

[CONTRIBUTION FROM THE EMERYVILLE RESEARCH CENTER OF THE SHELL DEVELOPMENT CO.]

The Preparation of Dialkyl Alkylphosphonates by Addition of Dialkyl Phosphites to Olefins

BY A. R. STILES, W. E. VAUGHAN AND F. F. RUST

RECEIVED AUGUST 21, 1957

A peroxide (or light) initiated addition of dialkyl phosphites to olefins is shown to be of considerable utility in the synthesis of dialkyl alkylphosphonates. Some reaction chain lengths have been calculated for three ratios of the reactants—1-octene and dibutyl phosphite. Both olefin and cumene retard the reaction.

The preparation of alkylphosphines by the free radical addition of phosphine to unsaturated compounds has been described in a previous communication.[1] This paper covers an extension of the above study to other compounds having a reactive P–H group, namely, the dialkyl phosphites. The addition of these esters to olefins can also be initiated by actinic radiation or decomposing peroxides and accordingly can likewise be presumed to be free radical in character.[2] The mechanism involves the addition of the phosphite radical to the double bond followed by hydrogen atom abstraction from the phosphite ester.

$$(R'O)_2OPH + R \longrightarrow (R'O)_2OP- + RH$$

$$(R'O)_2OP- + RCH{=}CHR \longrightarrow (R'O)_2OPCHRCHR$$

$$(R'O)_2OPCHRCHR + (R'O)_2OPH \longrightarrow (R'O)_2OPCHRCH_2R + (R'O)_2OP-$$

where R is H or an alkyl radical. A number of examples of this reaction are presented in Table I.

One of the more notable characteristics of the phosphite–olefin systems is the effect of reactant ratios upon reaction rate or, what is its equivalent, chain length. A variation in the reactant ratio not only changes the rate of conversion but also influences the composition of the product. Specifically (*cf.* Tables III and IV), a high concentration of dibutyl phosphite gives a kinetic chain length which is quite large, while with a low phosphite concentration (*cf.* Table II) the kinetic chain length is much less and, in addition, falls off quite rapidly as the reaction proceeds. The composition of the product was determined from the ratio of the consumption of olefin to the consumption of phosphite. In the experiment with a high concentration of olefin, the product contained between two and three olefin units to one phosphite unit, whereas with a low concentration of olefin in the starting material the product contained only slightly more than one olefin unit for every phosphite unit.

The kinetic chain length, which is a measure of peroxide efficiency, is computed from the number of molecules, both phosphite and olefin, reacted per initiating free radical. This latter value is twice the number of molecules of peroxide decomposed, which value in turn is derivable from the rate expression.[3]

(1) A. R. Stiles, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **74**, 3282 (1952).

(2) A. R. Stiles, D. Harman and F. F. Rust, U. S. Patent 2,724,718 (to Shell Development Co.), Nov. 22, 1955.

(3) J. H. Raley, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **70**, 88 (1948).

TABLE I

REACTION OF DIALKYL PHOSPHITES WITH OLEFINS

Initiated by 5 mole % di-*t*-butyl peroxide[b]

Olefin and phosphite[a]	Temp., °C.	Time, hr.	Product phosphonate	Conversion,[c] %	B.p. °C.	Mm.	$n^{20}D$	Phosphorus, % Found	Phosphorus, % Calcd.	Carbon, % Found	Carbon, % Calcd.
1-Hexene (1.2) Diethyl (0.7)	130	16	Diethyl hexyl- $(C_2H_5O)_2PO(C_6H_{13})$	29	126	10	1.4297	13.5	13.5	54.8	54.1
1-Octene (3.0) Dibutyl (1.0)[d]	25[d]	7	Dibutyl octyl- $(C_4H_9O)_2PO(C_8H_{17})$	54.5	146–152	1	1.4396	9.95	10.1	62.5	62.5
1-Decene (0.2) Dibutyl (0.1)	120	16	Dibutyl decyl- $(C_4H_9O)_2PO(C_{10}H_{21})$	25.2	157	1	1.4426	8.85	9.3	64.2	64.6
1-Tetradecene (1.0) Dimethyl (1)	160–190	1	Dimethyl tetradecyl- $(CH_3O)_2PO(C_{14}H_{29})$	61	142	MD[e]	1.4478	10.1	10.1	62.6	62.7
2-Butene (0.1) Dibutyl (0.2)	120	16	Dibutyl *sec*-butyl- $(C_4H_9O)_2PO(C_4H_9)$	77	143–146	13	1.4322	12.2	12.4	57.4	57.5
Cyclohexene (1.5) Dibutyl (3.1)[f]	170	2	Dibutyl cyclohexyl- $(C_4H_9O)_2PO(C_6H_{11})$	52	133–134	1	1.4544	11.1	11.2	60.8	60.8
Diisobutylene (0.2) Dibutyl (0.4)[g]	130	16	Dibutyl "isooöctyl-" $(C_4H_9O)_2PO(C_8H_{17})$	42	116	1	1.4377	10.5	10.1	61.9	62.7

[a] Moles in parentheses. [b] 5 mole % based on phosphite. [c] Based on the smaller component of the reaction mixture. [d] Acetone (9.8 mole % based on phosphite) as sensitizer in conjunction with radiation of G. E. AH_4 lamp. [e] Molecular distillation. [f] 2.5 mole % di-*t*-butyl peroxide. [g] 3.3 mole % di-*t*-butyl peroxide.

TABLE II

REACTION OF 3 MOLES OF 1-OCTENE AND 1 MOLE OF DIBUTYL PHOSPHITE

5 mole per cent. (on the phosphite) di-*t*-butyl peroxide; 120°

Time, hr.	$n^{25}D$	Amount of reaction: 1-Octene, mmoles/g.	Amount of reaction: Dibutyl phosphite, mmole/g.	Ratio olefin: phosphite reacted	Chain length
0	1.4130	0	0	..	
1	1.4146	0.36	.12	3.0	83
2	1.4156	.54	.18	3.0	42
3	1.4162	.66	.26	2.5	37
4	1.4169	.77	.29	2.7	25
5	1.4170	.78	.32	2.4	32
6	1.4182	.97	.41	2.4	32
7	1.4189	1.07	.44	2.4	28
8.5	1.4197	1.11[a]	.52[b]	2.1	17

[a] 19.6% conversion of 1-octene. [b] 28% conversion of dibutyl phosphite.

TABLE III

REACTION OF 1 MOLE OF 1-OCTENE AND 1 MOLE OF DIBUTYL PHOSPHITE

5 mole per cent. (on the phosphite) di-*t*-butyl peroxide; 120°

Time, hr.	$n^{20}D$	Amount of reaction: 1-Octene, mmoles/g.	Amount of reaction: Dibutyl phosphite, mmoles/g.	Ratio olefin: phosphite reacted	Chain length
0	1.4170	0	0	..	
1	1.4204	0.53	0.44	1.2	98
2	1.4232	0.96	0.61	1.6	61
3	1.4257	1.31	0.95	1.4	74
4	1.4287	1.68	1.26	1.3	73
5.1	1.4299	1.84	1.34	1.4	69
6	1.4332	2.37	1.76	1.4	69
7.2	1.4351	2.62	2.01	1.3	62
8.5	1.4378	2.94[a]	2.34[b]	1.3	54

[a] 95% conversion of 1-octene. [b] 73.5% conversion of dibutyl phosphite.

The implication of the foregoing is that the olefin is itself the inhibitor. This conclusion is supported by the series of experiments illustrated in Fig. 1. The most rapid reaction shown, between equimolar amounts of phosphite and 1-octene with diluent, is considerably reduced in extent when the octane is replaced with olefin and even more so when it is replaced with cumene. Thus, an olefin having an easily removable hydrogen atom in the allyl position gives a relatively unreactive allyl radical, which effectively interrupts the chain addition of dialkyl phosphites to olefins.

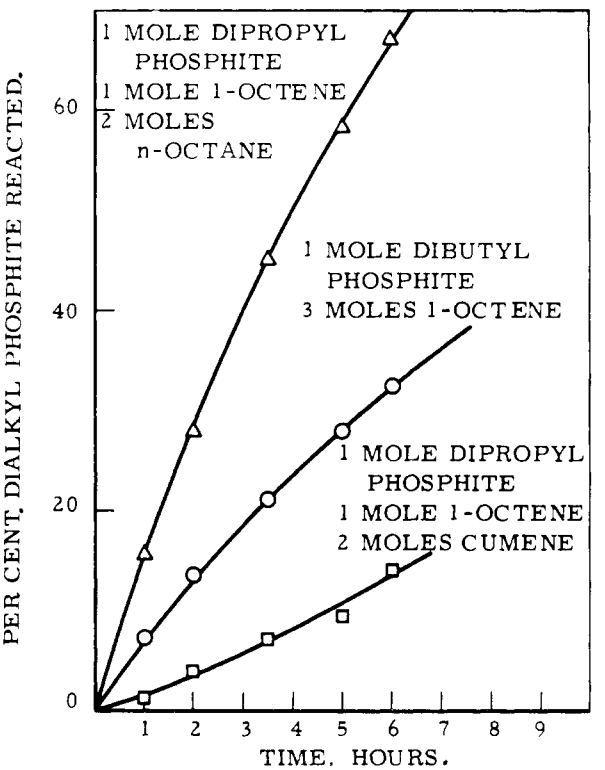

Fig. 1.—Addition of dialkyl phosphite to 1-octene.

Experimental

The various dialkyl phosphites were prepared by treating phosphorus trichloride with the desired alcohol according to the procedure of McCombie, Saunders and Stacey.[4] The 1-olefins were products of The Connecticut Hard Rub-

(4) H. McCombie, B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 380 (1945).

TABLE IV

REACTION OF 1 MOLE OF 1-OCTENE AND 3 MOLES OF DIBUTYL PHOSPHITE

5 mole per cent. (on the phosphite) di-*t*-butyl peroxide; 120°

Time, hr.	$n^{20}D$	Amount of reaction: 1-Octene, mmoles/g.	Dibutyl phosphite, mmoles/g.	Ratio olefin: phosphite reacted	Chain length
0	1.4201	0	0	..	
0.75	1.4275	1.23	0.96	1.3	221
1	1.4297	1.58	1.27	1.3	221
1.5	1.4297	1.82	1.27	1.5	
2	1.4300	1.73	1.26		
2.5	1.4298	1.73	1.27		
3	1.4300	1.81	1.30		
3.5	1.4300	1.70	1.32		
4	1.4302	1.79[a]	1.31[b]		

[a] 100% conversion of 1-octene. [b] 32% conversion of dibutyl phosphite.

ber Co.; the 2-butene and diisobutylene were obtained from Shell Oil Co. and the cyclohexene was Eastman Kodak Co. white label. All olefins were refractionated before use.

The experimental procedure was comparatively simple. The mixed reactants were heated at the desired temperatures either in sealed glass bombs or, if sufficiently high boiling, in open vessels. The product mixtures were then fractionated. In some cases the course of the reaction was followed by periodic sampling and analyses.

Analytical. The analysis of mixtures of dibutyl phosphite and olefin consists of (1) determining the neutral equivalent, which is the direct measure of the phosphite content and (2) determining the bromine number. The difference between the two values is then the measure of the olefin content.

(1) **Neutral Equivalent of Olefin–Dibutyl Phosphite Mixtures.**—A weighed sample (200–300 mg.) was dissolved in 25 ml. of 50% aqueous alcohol and allowed to react with excess 0.1 *N* alkali for 10 minutes and then titrated to the phenolphthalein end-point with 0.1 *N* acid.

(2) **Bromine Number of Olefin–Dibutyl Phosphite Mixtures.**—A weighed sample (200–400 mg.) was allowed to react for 45 min. with an excess of 0.1 *M* bromine in carbon tetrachloride in an atmosphere of dry nitrogen. At the end of this period the excess bromine was determined as previously described.

In Table V are contained the 1-octene and phosphite contents of several mixtures (all containing peroxide) used in rate studies. It will be noticed that as the phosphite concentration is increased, the reliability of the analysis for octene is decreased. This may be due to the large amount of HBr liberated when the phosphite is present in excess.

TABLE V

ANALYSIS OF STOCK SOLUTIONS

Ratio octene: phosphite	1-Octene, mmoles/g. Calcd.	1-Octene, mmoles/g. Found	Dibutyl phosphite, mmoles/g. Calcd.	Dibutyl phosphite, mmoles/g. Found
3:1	5.70	5.67	1.86	1.84
1:1	3.19	3.10	3.19	3.19
1:3	1.41	1.64	4.19	4.14

EMERYVILLE, CALIF.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

The Phosphopeptones Obtained from α-, β- and Whole Casein by Partial Hydrolysis with Pepsin[2]

BY M. L. GROVES, N. J. HIPP AND T. L. MCMEEKIN

RECEIVED AUGUST 23, 1957

Methods are described for fractionating the gel phosphopeptones obtained from partial peptic hydrolysates of α-, β- and whole casein. The phosphopeptone fraction prepared from β-casein, insoluble at *p*H 3.5, contained two electrophoretic components and a large proportion of the phosphorus of β-casein. These two components were separated by fractionation with ammonium sulfate. They were also prepared in good yield from whole casein by the same method.

Many investigators have studied the insoluble phosphopeptone (para- or pseudo-nuclein) formed during the early stages of the hydrolysis of casein by pepsin.[3–6] Holter, Linderstrøm-Lang and Funder[4] studied the rate of formation of the gelatinous insoluble phosphopeptone by means of viscosity and splitting of peptide bonds. The insoluble phosphopeptone was formed when the increase of amino nitrogen amounted to only 1.5% of the total nitrogen. They also found that the insoluble phosphopeptone had a nearly constant nitrogen-to-phosphorus ratio which was independent of the casein fraction used and the time of digestion. Mellander[6] has extensively reviewed the literature, has investigated the action of pepsin on cow's casein and human casein, and has found, in agreement with the literature, that the insoluble phosphopeptone is formed only from cow's casein.

The present investigation was undertaken in order to prepare large homogeneous phosphopeptones and to obtain information concerning the phosphorus linkage in casein. The isolation of the insoluble phosphopeptones from α-, β- and whole casein is described.

Materials and Methods

Samples of α-, β- and whole casein were prepared as previously described.[7] Crystalline pepsin was used. Nitrogen was determined by Nesslerization[8] and phosphorus by Sumner's[9] modification of the Fiske and Subbarow method.[10] All nitrogen and phosphorus values were corrected for 10% moisture. Zone electrophoretic determinations were made on Whatman No. 1 paper strips at 3° in a Durrum type cell using veronal buffer, 0.05/μ, *p*H 8.6. About 7.5 mg. of

(1) A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Dept. of Agriculture. Article not copyrighted.

(2) Presented at the 129th Meeting of the American Chemical Society, Atlantic City, N. J., September, 1956.

(3) R. H. Chittenden and H. M. Painter, *Trans. Conn. Acad. Arts Sci.*, **7**, 362 (1885–1888).

(4) H. Holter, K. Linderstrøm-Lang and J. B. Funder, *Compt. rend. trav. lab. Carlsberg*, **19**, No. 10, 1 (1933).

(5) K. Linderstrøm-Lang, *Ergebnisse Physiol.*, **35**, 415 (1933).

(6) O. Mellander, *Upsala Läkarefören. Förh.*, **52**, 107 (1947).

(7) N. J. Hipp, M. L. Groves, J. H. Custer and T. L. McMeekin, *J. Diary Sci.*, **35**, 272 (1952).

(8) F. C. Koch and T. L. McMeekin, THIS JOURNAL, **46**, 2066 (1924).

(9) J. B. Sumner, *Science*, **100**, 413 (1944).

(10) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).