compounds act as microbicidal or microbiostatic agents.

The final concentrations of solids used to treat blood were chosen according to their solubilities in undiluted blood. Sorbic acid, D-isoascorbic acid, and benzoic acid added without pH adjustment to the blood were not effective as preservatives [Table I (A)]. Propyl gallate displayed moderate effectiveness at a concentration of 0.35 g/100 mL. Of the solids screened in this investigation, only succinic acid (0.75 g/100 mL) and sodium polyphosphate (sodium hexametaphosphate) or sodium bisulfite (0.8–1.0 g/100 mL) are effective. We have performed experiments (data not shown) on a wide variety of dilution conditions with several blood samples, and we conclude that the use of sodium polyphosphate or sodium bisulfite provides the cheapest and most effective form of blood preservation. However, neither sodium polyphosphate nor sodium bisulfite completely prevents the degradation of blood protein. At effective doses of 0.8–1.0 g/100 mL for sodium polyphosphate and sodium bisulfite, the nonprotein nitrogen level of the blood increases ~250% compared to the nonprotein nitrogen level of fresh blood. At very low concentrations of succinic acid, there appeared to be an increase in the breakdown of blood protein compared to that of the control. The mechanism by which low concentrations of a succinic acid may stimulate blood protein breakdown is not clear.

The enhanced effectiveness of succinic acid at higher concentrations may reflect a pH effect. Sodium succinate, when tested at concentrations up to 1.4 g/100 mL, was not an effective blood preservative (data not shown). In order to determine whether pH alone can preserve blood protein, we incubated blood with varying concentrations of the inorganic acids H_2SO_4 and H_3PO_4 and the organic acids acetic acid and propionic acid. Even at moderate concentrations these acids cause blood to congeal at about pH 4.0. In some cases the blood does not congeal immediately but does so after some period of incubation. Thus, the problem of blood coagulation necessitates constant stirring when adding acid to produce a pH below 4.0 (Akers, 1973). Although packers may be able to stir blood continuously at collection, renderers must often wait for delivery of blood which has been stored without stirring for up to 48 h. In our experiments, we arbitrarily discarded from consideration as a blood preservative any acid (or other chemical) which caused clumping or congealing of blood during 48 h of incubation, without stirring, at 35 °C.

Acetic acid (99.5% w/v), phosphoric acid (85% w/v), sulfuric acid (96%), and propionic acid were added directly to whole blood to determine their effectiveness as blood preservatives. Over a 48-h incubation period, no tested concentration of H_2SO_4 (0.5 g/100 mL or greater), added as an undiluted acid, is an effective blood preservation agent which does not cause blood to congeal. Phosphoric acid, at a final concentration of 0.7 g/100 mL, is a very good blood preservative as are acetic acid (0.5 g/100 mL) and propionic acid (0.75 g/100 mL) [Table I (B)].

In summary, due to the problems inherent in using acids and the apparent unacceptability of sodium bisulfite, we recommend that sodium polyphosphate be used to preserve raw industrial animal blood for periods up to 2 days before processing into animal feeds.

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Synthesis of *O,O*-Dimethyl 2,2-Dichloro-1-(acyloxy)ethenephosphonates, Major Constituents in Acylated Trichlorfons

In the synthesis of acylated trichlorfons, the dehydrohalogenated compounds are the major contaminants. Six of the compounds were synthesized by using 1,5-diazabicyclo[5.4.0]undec-5-ene (BDU) as the dehydrohalogenation reagent. The compounds were synthesized for structural confirmation and for assessment of their contribution to the toxicity of the parent compounds that are used as experimental insecticides on forest insect pests.

Acyl trichlorfons (I) enjoy some success in insect control (Casida and Arthur, 1959; Pieper and Richmond, 1976). They have superior solubility properties in ordinary solvents compared with the nonacylated trichlorfon and their low mammalian to high insect toxicity ratios are environmentally advantageous in forest applications. In a study with a homologous series of acyl trichlorfons, it was found that their syntheses were accompanied by large amounts (10% or more) of impurities with absorption in the 6.32-μm region of the infrared spectrum. These impurities were identified as the dehydrohalogenated acyl trichlorfons, *O,O*-dimethyl 2,2-dichloro-1-(acyloxy)ethenephosphonates (III) and their structural verification by synthesis (Figure 1) is described.

The impurities were difficult to separate from the acylated trichlorfons, especially in the scale needed in an experimental spray operation. Dehydrohalogenated compounds can be removed from acylated trichlorfons by vacuum distillation; however, the acylated trichlorfons undergo extensive decomposition in the process.

Table I. O,O-Dimethyl 2,2-Dichloro-1-(acyloxy)ethenephosphonates Synthesized and Elemental Analysis

compd III RCO	empirical formula	elemental analysis, calcd C	calcd H	found C	found H	bp, °C/mmHg	yield, %
acetyl	$C_6H_9Cl_2O_5P$	27.40	3.45	27.29	3.45	70/0.2	71
1-oxopropyl	$C_7H_{11}Cl_2O_5P$	30.35	4.00	30.94	4.09	90/0.1	63
1-oxobutyl	$C_8H_{13}Cl_2O_5P$	33.01	4.50	32.84	4.47	60/0.05	NA
1-oxohexyl	$C_{10}H_{17}Cl_2O_5P$	37.63	5.37	37.82	5.60	70/0.07	NA
1-oxononyl[a]	$C_{12}H_{23}Cl_2O_5P$					120/0.05	65
1-oxododecyl	$C_{15}H_{29}Cl_2O_5P$	47.65	7.25	47.99	7.26	165/0.3	32

[a] Elemental analysis was not conducted; the structure was determined by infrared spectrum correlation.

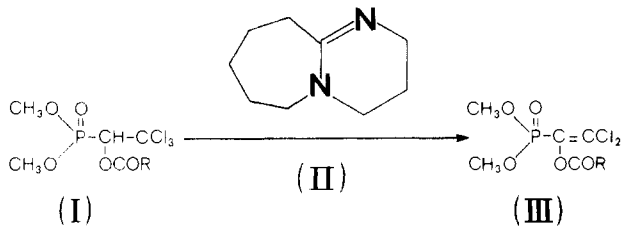

Figure 1

In 1971, field tests were conducted against the western spruce budworm (*Choristoneura occidentalis* Freeman) with 1-oxododecyl trichlorfon (I; R = $C_{11}H_{23}$) contaminated with the dehydrohalogenated 1-oxododecyl trichlorfon (III; R = $C_{11}H_{23}$) (Pieper and Richmond, 1976). Concern for the possible biological activity of this and other dehydrohalogenated derivatives made it necessary to synthesize pure dehydrohalogenated acyl trichlorfons for biological evaluation and, ultimately, to assess their contribution to the toxicity of the parent compounds.

Although it is difficult to synthesize pure acylated trichlorfons from trichlorfon, it is relatively easy to synthesize pure dehydrohalogenated acylated trichlorfons from impure acylated trichlorfons. Six dehydrohalogenated acylated trichlorfons were synthesized, and none showed any biological activity in laboratory screening experiments with the western spruce budworm (Robertson et al., 1978). The reagent of our choice for dehydrochlorination of acylated trichlorfons is 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU = II). The usual dehydrochlorination reagents, such as dimethylaniline, triethylamine, and pyridine, give poor or no yields with this reaction.

MATERIALS AND METHODS

The six dehydrohalogenated compounds synthesized in this investigation were acetyl (Arthus and Casida, 1957), 1-oxopropyl, 1-oxobutyl, 1-oxohexyl, 1-oxononyl, and 1-oxododecyl derivatives (Table I). Boiling points of the derivatives were approximate because of the nature of the distilling apparatus (Kontes K548250) and quantities of material; however, all the derivatives were distilled without decomposition. The infrared spectrum of each dehydrohalogenated compound was identical with that of the impurity found in the corresponding acylated trichlorfon. Trichlorfon was furnished by the Mobay Chemical Corp., Kansas City, MO. Acylated trichlorfons were prepared by acyl chloride acylations of trichlorfon (Fricke, 1965) and were purified by column chromatography on silica gel using cyclohexane and acetone (1:1). Subsequently it was found that purification was unnecessary for the synthesis. The DBU was purchased from Aldrich Chemical Co., Milwaukee, WI. Elemental analyses were performed by Elek Microanalytical Laboratories, Torrance, CA. The following example is the general method of synthesis.

O,O-Dimethyl 2,2-Dichloro-1-(1-oxododecyl)-ethenephosphonate (IV). 1-Oxododecyl trichlorfon (5 g, 0.011 mol) in 20 mL of tetrahydrofuran was cooled in an ice bath. To the solution was added 1.8 g (0.012 mol) of 1,5-diazabicyclo[5.4.0]undec-5-ene. The resulting solution was stirred and allowed to warm to room temperature overnight. The mixture was diluted with 200 mL of water and extracted with methylene chloride. The extract was washed, in turn, with water, 1 N hydrochloric acid, water saturated with sodium chloride, and water. The organic phase was dried with anhydrous magnesium sulfate and the solvent was evaporated, leaving an oil that was distilled at 165 °C/0.3 mmHg to give 1.4 g (32%) of O,O-dimethyl 2,2-dichloro-1(1-oxododecyl)ethenephosphonate: IR (film on potassium bromide) 3.42, 3.51, 5.67, 6.15, 6.32, 6.88, 7.86 μm; M_r calcd, 403.3; found, 403, by vapor pressure osmometer. Anal. Calcd for $C_{16}H_{29}Cl_2O_5P$: C, 47.65; H, 7.25. Found: C, 47.88; H, 7.26.

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