87923-95-3; V-B, 2486-71-7; V-C, 635-21-2; V-D, 2486-70-6; VI-B, 87923-93-1; VI-C, 26208-56-0; VI-D, 80029-31-8; CH_3NHCHO, 123-39-7; SCl_2, 10545-99-0.

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Synthesis and Biological Activity Studies of Selected Organophosphorus Esters

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Thirty organophosphorus esters were synthesized and evaluated as possible insecticide synergists against boll weevils, *Anthonomous grandis*. B-Esterase and acetylcholinesterase activity from organophosphorus-susceptible weevils were measured spectrophotometrically with *S*-phenyl thiobenzoate and acetylthiocholine as substrates. The structure–biological activity relation may be divided into three major effects—a lipophilic effect, an electronic effect, and a steric effect. In vitro and in vivo inhibition and toxicity data support the hypothesis that synergism of methyl paraoxon results from the inhibition of *S*-phenyl thiobenzoate hydrolyzing esterase by selected organophosphorus esters.

The most thoroughly studied and best known examples of organophosphorus synergism are those involving carboxylesterase inhibitions with malathion (Frawley et al., 1957; Murphy et al., 1959; Eto and Casida, 1962; Eto et al., 1965; Casida, 1961). These B-esterase enzymes hydrolyze the α-carboxyl ester group of malathion to yield the α-monoacid and/or the β-ester group to the β-monoacid, both very weak cholinesterase inhibitors (Cook et al., 1958). Because the low mammalian toxicity of malathion is the result of its degradation by carboxylesterase, the inhibition of this enzyme brings about a subsequent increase in toxicity.

DEF (*S,S,S*-tributyl phosphorotrithioate) was found to synergize methyl parathion and methyl paraoxon against boll weevils, *Anthonomous grandis* (Chambers, 1979a). Additionally, DEF has been found to synergize the carbamate and organochlorine insecticides when applied to certain resistant insect strains (Sun et al., 1967). Striking differences in the patterns of synergisms of organophosphorus, carbamate, and pyrethroid insecticides in boll weevils by DEF, and by piperonyl butoxide and sesamex, suggest that the DEF-induced synergism is not via mixed-function oxidase inhibition (Wilkinson, 1976). Rather, inhibition of β-esterases appears to be the mechanism involved, though the role of these enzymes in limiting insecticide toxicity has not been fully explained (chambers, unpublished data). Recently, mixtures of carbamate–organophosphorus derivatives have been shown to have synergistic possibilities (Bakunick, 1979).

In this paper, the preparation of organophosphorus esters (structurally similar analogues of DEF) and their synergistic activity toward methyl paraoxon are reported.

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EXPERIMENTAL SECTION

Methods and Materials. Column adsorption chromatography (Brinkman silica gel 60) was used to purify all ester compounds except the *o*-phenylene phosphorothioates (distill). An ether–hexane (1:1) solvent system was used. Compound purity was checked by thin-layer chromatography using Eastman Chromagram precoated sheets (13181 silica gel).

All nuclear magnetic resonance spectra (^1H NMR) were obtained at 60 MHz by using a Varian A-60 nuclear magnetic resonance spectrometer. Tetramethylsilane was used as the internal standard and 99.8% chloroform-*d* was used as the solvent. All mass spectroscopy data were obtained by using a Hewlett-Packard Model 5930 quadrupole electron impact mass spectrometer. All infrared spectra were obtained by using a Perkin-Elmer Model 283 grating infrared spectrophotometer. The spectra of liquids were taken as films formed between two cesium iodide plates; potassium bromide was used in preparing pellets of solid samples.

Synthesis. The analytical data are given in Table I. The IR, ^1H NMR, and MS spectra were consistent with the proposed structure (see supplementary material for a listing of the data; see paragraph at end of paper regarding supplementary material).

S,S,S-Trialkyl Phosphorotrithioates (**1–5**). One equivalent of phosphorus trichloride was alkylated with a mixture of 3 equiv of the appropriate mercaptan and 3 equiv of triethylamine in benzene at 0 °C (Ford-Moore and Perry, 1963). After the reaction mixture was warmed to room temperature and stirred for 12 h, triethylammonium hydrochloride was removed by filtration. Unreacted phosphorus trichloride and benzene were removed by using a rotary evaporator. One equivalent of the *S,S,S*-trialkyl

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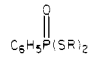
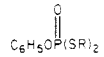
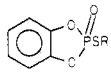
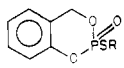
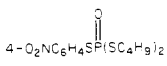
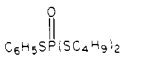
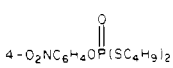
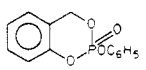
Table I. Analytical Data for the Organophosphorus Esters

structure	compound no.	R	% yield[a,b]	TLC R_f[c]
$(RS)_3PO$	1	-n-C_3H_7	78.4	0.87
	2	-i-C_3H_7	66.5	0.85
	3	-n-C_4H_9	82.7	0.85
	4	-i-C_4H_9	73.9	0.88
	5	-n-C_8H_{17}	65.4	0.93
	6	-n-C_3H_7	83.1	0.59
	7	-i-C_3H_7	67.4	0.59
	8	-n-C_4H_9	78.6	0.63
	9	-i-C_4H_9	52.1	0.64
	10	-t-C_4H_9	20.5	0.70
	11	-n-C_5H_{11}	82.0	0.71
	12	-i-C_5H_{11}	77.5	0.70
	13	-n-C_7H_{15}	59.7	0.74
	14	-n-C_8H_{17}	49.0	0.76
	15	-n-C_9H_{19}	41.7	0.77
	16	-n-C_3H_7	79.2	0.85
	17	-i-C_3H_7	64.6	0.87
	18	-n-C_4H_9	81.0	0.88
	19	-i-C_4H_9	76.6	0.83
	20	-n-C_3H_7	57.8	0.60
	21	-n-C_4H_9	60.0	0.69
	22	-n-C_3H_7	59.3	0.40
	23	-i-C_3H_7	58.8	0.38
	24	-n-C_4H_9	67.7	0.48
	25	-i-C_4H_9	60.2	0.53
	26	$-C_6H_5$	46.0	0.52
	27		38.3	0.81
	28		66.5	0.78
	29		92.2	0.83
	30		51.2	0.49

[a] All compounds were obtained as liquids except 26 (mp 86–87 °C) and 30 (mp 78–80 °C). [b] Elemental analysis (Galbraith Laboratories, Inc., Knoxville, TN) was obtained for compounds 8, 18, 21, and 24. An authentic sample of $(BuS)_3PO$ (Chemagro) was obtained for comparative structural assignments of the tributyl phosphorodithioates. [c] Solvent system was hexane–ether (1:1) for compounds 1–30 except it was butanol–acetic acid–water (5:2:3) for compounds 20–21.

phosphorotrithioite was oxidized with 1 equiv of 30% hydrogen peroxide in acetone and the solution was refluxed for 3–4 h (Stuebe et al., 1955). The crude phosphorotrithioates were added to water and extracted with ether. The ethereal extract was purified to yield the phosphorotrithioates.

S,S-Dialkyl Phenylphosphonodithioates (**6–15**). One equivalent of phenylphosphonodichloridate (Aldrich) was reacted with a mixture of 2 equiv of the appropriate mercaptan and 2 equiv of triethylamine in benzene at 0 °C (Arbuzov and Razumova, 1945). After being refluxed for 12 h, the mixture was purified to yield the phosphonodithioates.

S,S-Dialkyl O-Phenyl Phosphorodithioates (**16–19**). One equivalent of *O*-phenyl phosphorodichloridate (Aldrich) was reacted with a mixture of 2 equiv of the appropriate mercaptan and 2 equiv of triethylamine in benzene at 0 °C (Arbuzov and Razumova, 1945). After being warmed to room temperature, the solution was refluxed for 12 h. Workup and purification of the product yielded the phosphorodithioates.

S-Alkyl O-Phenylene Phosphorothioates (**20–21**). One equivalent of *S*-alkyl phosphorodichlorodichloridothioate was added through an addition funnel to a three-neck flask containing approximately 1 equiv (10% excess) of catechol and 2 equiv of pyridine in benzene at 0 °C (Kobayashi et al., 1969). The reaction mixture was allowed to warm to room temperature and then refluxed for 6–12 h. The organic salt was removed by filtration, the solvent was removed, and the ester was used in a crude state.

S-Alkyl Saligenin Cyclic Phosphorothioates (**22–25**). One equivalent of the appropriate *S*-alkyl phosphorodichloriothioate was added to a flask containing a mixture of 1 equiv of salicyl alcohol and 2 equiv of pyridine in chloroform (Kobayashi et al., 1969). The reaction was allowed to proceed for 6–8 h at room temperature. Workup and purification yielded the cyclic phosphorothioates.

S-Phenyl Saligenin Cyclic Phosphorothioate (**26**). One equivalent of the *S*-phenyl phosphorodichloridothioate was added to the 1 equiv of salicyl alcohol and 2 equiv of pyridine in chloroform at 0 °C (Kobayashi et al., 1969). The reaction mixture was allowed to warm to room temperature and heated at 30–35 °C for 6–12 h. Workup and recrystallization of the crystalline material from ethyl ether–petroleum ether gave the phenyl saligenin phosphorothioate.

S,S-Dibutyl S-(4-Nitrophenyl) Phosphorotrithioate (**27**) *and S,S-Dibutyl S-Phenyl Phosphorotrithioate* (**28**). To 1 equiv of potassium 4-nitrothiophenolate in 1,2-dimethoxyethane 1 equiv of *S,S*-dibutyl phosphorochloridithioate was added (Schrader, 1963). The contents in the flask were maintained at 0 °C during the initial addition and held at room temperature for 6 h. The crude trithioate was washed with water and passed through a silica gel 60 column to remove unreacted phenolic materials.

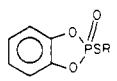
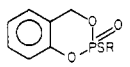
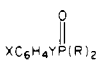
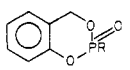
Similarly, potassium thiophenolate in 1,2-dimethoxyethane was reacted with the *S,S*-dibutyl phosphorochloridodithioate to form *S,S*-dibutyl *S*-phenyl phosphorotrithioate. Workup and purification yielded the phenylphosphorotrithioate.

S,S-Dibutyl 4-Nitrophenyl Phosphorodithioate (**29**). One equivalent of 4-nitrophenyl phosphorodichloridate (Aldrich) was added through an addition funnel to a flask containing a mixture of 2 equiv of butyl mercaptan and 2 equiv of triethylamine at 0 °C (Murphy et al., 1959; Eto and Oshima, 1962). The mixture was refluxed for 12 h. Workup and purification yielded the 4-nitrophenyl phosphorodithioate.

O-Phenyl Saligenin Cyclic Phosphate (**30**). One equivalent of *O*-phenyl phosphorodichloridate (Aldrich) was added to 1 equiv of salicyl alcohol and 2 equiv of pyridine in chloroform at 0 °C (Kobayashi et al., 1969). The reaction mixture was warmed to room temperature and then the solution was stirred for 6–12 h. Workup and recrystallization of the crystalline material from ethyl ether–petroleum ether yielded the saligenin cyclic phosphate.

Biological Activity Tests. In vitro carboxylesterase activities were obtained by using a 5 mg/mL homogenate of adult boll weevils according to the general method of Chambers (1973b). Weevils were homogenized (Ten Broeck homogenizers) in 0.8 M sucrose and the homogenate were centrifuged and filtered. All esterase assays were performed in 0.1 M, pH 7.4, Tris [2-amino-2-(hydroxymethyl)-1,3-propanediol] buffer (Sigma Chemical Co.). The substrate, *S*-phenyl thiobenzoate, dissolved in ethanol was used at a concentration of 0.025 M. A sample

Table II. In Vitro I_{50} Values against Boll Weevil B-Esterase (Substrate = *S*-Phenyl Thiobenzoate)

structure	compound no.	substituent R	I_{50} (M × 10^8)	r^a
$(RS)_3PO$	1	-*n*-C_3H_7	79.4	0.991
	2	-*i*-C_3H_7	900.0	0.999
	3	-*n*-C_4H_9	56.0	0.994
	4	-*i*-C_4H_9	100.0	0.998
	5	-*n*-C_8H_{17}		
	6	-*n*-C_3H_7	44.2	0.996
	7	-*i*-C_3H_7	110.0	0.995
	8	-*n*-C_4H_9	14.2	0.996
	9	-*i*-C_4H_9	31.2	0.999
	10	-*t*-C_4H_9	200.0	0.997
	11	-*n*-C_5H_{11}	9.73	0.984
	12	-*i*-C_5H_{11}	10.8	0.999
	13	-*n*-C_7H_{15}	4.84	0.995
	14	-*n*-C_8H_{17}	14.6	0.971
	15	-*n*-C_9H_{19}	60.7	0.966
	16	-*n*-C_3H_7	16.7	0.990
	17	-*i*-C_3H_7	300.0	0.997
	18	-*n*-C_4H_9	15.5	0.999
	19	-*i*-C_4H_9	300.0	0.992
	20	-*n*-C_3H_7	2500	0.988
	21	-*n*-C_4H_9	200	0.999
	22	-*n*-C_3H_7	0.182	0.998
	23	-*i*-C_3H_7	0.152	0.996
	24	-*n*-C_4H_9	0.124	0.967
	25	-*i*-C_4H_9	0.144	0.999
	26	-C_6H_5	0.184	0.976
		substituent		
		X Y R		
	27	4-NO_2 S -SC_4H_9	0.618	0.994
	28	H S -SC_4H_9	6.18	0.997
	29	4-NO_2 O -SC_4H_9	0.088	0.998
		substituent R		
	30	-OC_6H_5	0.306	0.983

[a] *r* = correlation coefficient based on quadruple assays.

Table III. In Vivo Inhibition of Carboxylesterase and Synergism of Methyl Paraoxon in the Boll Weevil by Selected Phosphorus Esters

treatment compound[a]	carboxylesterase data		toxicity data		synergism ratio
	sp act.[b]	% inhibn ± SD	obsd mortality, treated/control[c]	calcd dose,[d] treated/control	
7	1.89 (3)	67.0 ± 10.5	62.4/50.7	4.36/3.71	1.17
14	1.43 (3)	74.2 ± 4.1	64.4/50.7	4.48/3.71	1.21
24	2.06 (3)	46.8 ± 4.0	36.6/35.6	3.06/2.83	1.08
29	0.86 (5)	84.7 ± 4.6	67.4/31.3	4.72/2.81	1.73
3	1.90 (1)	76.9			
8	1.55 (1)	80.5			
10	1.12 (1)	79.9			
18	1.46 (1)	81.6			

[a] All compounds tested at 10 nmol/g of diet. [b] Nanomoles of substrate hydrolyzed per minute per milligram protein: mean of the number of determinations given in parentheses. [c] All insects received methyl paraoxon (3 μg/g) topically; controls received no treatment via the diet. Pooled data of two replications. [d] Calculated from dosage-mortality (log-probit) regression line for methyl paraoxon.

of homogenate receiving absolute ethanol (10 μL/mL homogenate) in lieu of substrate served as a blank. Nonenzymic hydrolysis was previously found to be negligible. In vitro inhibition (Table II) was determined on serial dilutions of the test compounds by incubating each with the enzyme for 15 minutes prior to assay of residual enzyme activity. All absorbance measurements were made on a Beckman Model 3600 spectrophotometer at 412 nm. I_{50} values were obtained by linear regression analysis of plots of percent inhibition vs. the logarithm of concentration of inhibitor solution.

In vitro acetylcholinesterase inhibition was determined by using a 4 mg/ml concentration of boll weevil homogenate (Ellman et al., 1961). The substrate used was 10^{-3} M acetylthiocholine. A sample incubated with eserine (10^{-5} M) was used to correct for nonenzymic hydrolysis. The percent inhibition was measured for all compounds at 10^{-4} M, and the time of inhibition was 15 min. I_{50} values were obtained only for the saligenin compounds.

In vivo carboxylesterase activities (Table III) were obtained by using a diet containing the inhibitor fed to boll weevils. An artificial diet was prepared as developed by

the U.S. Department of Agriculture Boll Weevil Rearing Laboratory. Test compounds were added and the concentrations (10^{-7}–10^{-5} M) used were selected from preliminary tests. Weevils were starved for 1 day and then fed the treated diet for 1 day. Weevils receiving an untreated diet served as controls.

For in vivo enzyme assay, 24 weevils from each treatment group were homogenized in 0.8 M sucrose, centrifuged, filtered, and then assayed in Tris buffer as for in vitro studies. Ethanol (10 μL/mL homogenate) was added to assay tubes and the solution was incubated for 15 min prior to measurement of enzyme activity. Carboxylesterase activity was determined for each homogenate by using 0.025 M *S*-phenyl thiobenzoate as the substrate. A fourth aliquot, preincubated with 3×10^{-6} M 2-phenoxy-4*H*-1,3,2-benzodioxaphosphorin 2-oxide served as a blank to correct for nonenzymic hydrolysis in assay samples. Protein was determined by the method of Lowry et al. (1951) using an aliquot of homogenate equivalent to 2 mg wet weight. Specific activities (nanomoles per minute per milligram protein) and percent inhibition were then calculated.

Four compounds were selected for synergism studies, representing a range of about 45–85% in vivo inhibition. The potential synergist was incorporated into the diet at 10^{-5} mol/kg and fed to weevils for day prior to bioassay. Weevils fed untreated diet served as a reference group. Weevils from each treatment and control group were used for enzyme assay, after being held on a selected inhibitor diet and control diet for 24 h. Methyl paraoxon (3 μg/g of body weight) was applied topically by using acetone as the solvent. A control group received an equal volume of acetone. Each assay consisted of 40 control insects and 80 treated insects, and each assay was replicated twice. The dosages were based on the average weight (0.015 g) for boll weevils. All insects were then fed on regular diet after treatment with insecticide. Percent mortality was determined 48 h posttreatment and the corrected percent mortality (% Mc) was obtained by the method of Abbott (1925). The % Mc were then converted to probit mortality values (Y). A previously established plot of probit mortality vs. log dosage for methyl paraoxon against boll weevils (48-h mortality) gave a Y intercept (Y_0) of 2.5724 and a slope (b) of 4.2913 ($r > 0.999$). From these data, the calculated insecticide dosage required to yield the observed mortalities were determined from eq 1:

$$\text{calculated dosage} = \text{antilog}\,[(Y' - Y_0)/b] \quad (1)$$

Y' was the probit of the corrected mortality.

By use of the calculated dosages, the synergistic ratio (SR) could be determined by eq 2:

$$\text{SR} = \frac{\text{calculated dosage (synergist treated)}}{\text{calculated dosage (no synergist)}} \quad (2)$$

Thus, an SR value greater than 1 indicates synergism and an SR equal to 1 indicates no effect.

RESULTS AND DISCUSSION

Biological activity studies in vitro (Table II) showed an apparent qualitative correlation between esterase activity and chemical structure of inhibitor. Since organophosphate acetylcholinesterase inhibition (Eto, 1974) is irreversible, the organophosphate esters used in this study would seem likely to inhibit irreversibly the enzyme which splits *S*-phenyl thiobenzoate.

The four compounds tested were *S*,*S*-diisopropyl phenylphosphonodithioate (**7**), *S*,*S*-dioctyl phenylphosphonodithioate (**14**), *S*-butyl saligenin cyclic phosphorothioate (**24**), and *S*,*S*-dibutyl *O*-(4-nitrophenyl) phosphorodithioate (**29**). The degree of synergism for each of the four compounds increased as the in vivo esterase inhibition increased.

In in vitro acetylcholinesterase inhibition studies, only compound 30 was effective at less than 10^{-5} M (I_{50} = 5 μm). Other saligenin cyclic phosphates (**22–25** and **26**) were weak inhibitors with I_{50}'s ranging from 26 to 317 μM. No other compounds examined yielded 50% inhibition of acetylcholinesterase at the highest concentrations tested (100 μM).

In in vitro studies, carboxylesterase activity varied significantly with the particular substituents surrounding the phosphorus moiety. For example, the *S*,*S*,*S*-trialkyl thioates (**1–5**) showed the lowest carboxylesterase inhibition of any series whereas the saligenin derivatives gave substantial inhibition. The *S*-butyl esters of all the compounds appreciably inhibited carboxylesterase activity.

At least one compound was selected from each structural class except the *O*-phenylene phoshorothioates for in vivo studies. These studies wee used to determine if the compound is absorbed and retained by the insect in sufficient concentration to inhibit the enzymes in the live insect.

Table III gives inhibition and synergism data for the compounds that were studied in vivo. Concentrations of 10^{-7} M gave very little carboxylesterase inhibition, whereas 10^{-5} M diet solutions gave 47–85% inhibition for the compounds tested. *S*,*S*-Dibutyl *O*-(4-nitrophenyl) phosphorodithioate (**29**), which gave the greatest in vitro inhibition, was also found to exhibit consistently the highest in vivo inhibition. The para electron withdrawing nitro group on the phenyl ring accounts for the increased activity. In contrast, the *S*-butyl saligenin cyclic phosphorothioate (**24**) (high in vitro inhibition) gave the lowest in vivo values. Possibly, this compound underwent hydrolysis or other degration before it had time to phosphorylate the esterase. Also, those sterically defined compounds containing bulky alkyl groups, *S*,*S*-di-*tert*-butyl phenylphosphonodithioate (**10**) and *S*,*S*-diisopropyl phenylphosphonodithioate (7), which gave low in vitro values were found to give relatively good in vivo inhibition. *S*,*S*-Dioctyl phenylphosphonodithioate (**14**) characteristic of lipophilic behavior gave high in vivo inhibition comparable to 4-nitrophenyl phosphorodithioate (**29**). The stability of the phosphonate derivatives (C–P bonding) may be the most significant factor relating to the relatively good in vivo potentiations. Cholinesterase inhibition in vivo was found to be virtually nonexistent at the synergist dosage levels. In our desire to correlate structure–activity relationships in vivo to our synergist application with the insecticide, methyl paraoxon, we chose four compounds (**7, 14, 24, 29**) that cover the steric, electronic, and lipophilic parameters. Additionally, two compounds (**7, 14**) were chosen from the same class and all except the saligenin derivative (**24**) showed high in vivo inhibition. These studies were used to determine the relative affinity of the insecticide for carboxylesterase (*S*-phenyl thiobenzoate–esterase) and to predict how well a synergist will work with a given insecticide.

From the synergism data tabulated in Table III, the 4-nitrophenyl phosphorodithioate (**29**) proved to be the best synergist with an SR value of 1.73. The lipophilic compound, *S*,*S*-dioctyl phenylphosphonodithioate (**14**) gave the second best synergistic ratio. And finally, the *S*-butyl saligenin cyclic phosphorothioate (**24**) gave virtually no synergism, which correlated well with the low in vivo inhibitions.

In summary, the structure–activity studies suggest that (1) in vitro inhibition by a compound does not ensure in

vivo activity at least when administered in the diet, (2) in vivo inhibition correlates well with synergism of methyl paraoxon, (3) lack of antiacetylcholinesterase activity by most compounds make them useful tools for selective inhibition of certain B-esterases, and (4) the degree of synergism is too low to be of economic value in boll weevil control.

Registry No. 1, 1642-44-0; 2, 85480-01-9; 3, 78-48-8; 4, 78788-15-5; 5, 3819-72-5; 6, 68598-42-5; 7, 68598-41-4; 8, 2797-64-0; 9, 68598-40-3; 10, 68598-39-0; 11, 68598-38-9; 12, 68598-37-8; 13, 85480-02-0; 14, 85480-03-1; 15, 85480-04-2; 16, 68598-36-7; 17, 85480-05-3; 18, 68598-35-6; 19, 85480-06-4; 20, 85480-07-5; 21, 85480-08-6; 22, 26115-85-5; 23, 26115-86-6; 24, 24067-01-4; 25, 85480-09-7; 26, 24067-02-5; 27, 85480-10-0; 28, 30299-04-8; 29, 85480-11-1; 30, 4081-23-6; methyl paraoxon, 950-35-6; B-esterase, 9016-18-6.

Supplementary Material Available: A listing of data from IR and ^{1}H NMR spectra of esters 1–30 and MS of esters 3, 8, 18, 21, and 24 (7 pages). Ordering information is given on any current masthead page.

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Quantitation of Free and Hydrolyzable Phenolic Acids in Seeds by Capillary Gas–Liquid Chromatography

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Free phenolic acids were initially extracted with tetrahydrofuran, followed by extraction of soluble phenolic esters with methanol–acetone–water. Alkaline hydrolysis was employed to release aglycons from soluble phenolic esters (2 N NaOH) and residue (4 N NaOH). The Me_3Si derivatives of the released phenolic acids were quantitated by capillary gas–liquid chromatography within an elution time of 20 min. Greater precision was achieved by using methyl heptadecanoate instead of *n*-tetracosane as the internal standard. Although operations were conducted in the dark under nitrogen, recoveries of *p*-hydroxybenzoic, vanillic, and syringic acids exceeded 90%, but those of *p*-coumaric, ferulic, and sinapic acids were 87, 82, and 78%, respectively, and that of caffeic acid was only 17%.

Phenolic compounds occur widely as microconstituents in plant foods and there is increasing interest in their effects on food quality. Among the various forms of phenolic compounds, the free phenolic acids, esters, and glycosides that contain an acrylic acid group conjugated with the aromatic ring are of particular concern. These monocyclic phenolics are readily oxidized, leading to the formation of quinones which further react to form polymers or bind to proteins and carbohydrates (Sabir et al., 1974; Van Sumere et al., 1975). The *o*-dihydroxyphenols such as caffeic acid are particularly reactive and, thus, are found in only low concentrations in seeds (Sosulski, 1979). However, esters of these phenolic acids, such as chlorogenic acid (3-caffeoylquinic acid), occur widely in plant tissues and represent 3–4% of the defatted flour from sunflower. Sabir et al. (1974) demonstrated that, at neutral pH, one-third of the chlorogenic acid in slurries of sunflower flour was covalently bonded to low molecular weight proteins and polypeptides. Under alkaline conditions, oxidation and bonding of chlorogenic acid with sunflower proteins may be extensive, developing dark green colors that oxidize further to a dark brown on prolonged storage.

Simple phenolic acids have been characterized as having sour, bitter, astringent, and phenol-like flavors (Arai et al., 1966; Maga and Lorenz, 1973). However, it is the choline ester of sinapic acid, sinapine, which occurs in concentrations of 0.5–1.5% in defatted crambe (Austin and Wolff,

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