Synthetic Analogues of Phytoalexins. Synthesis and Antifungal Activity of Potential Free-Radical Scavengers

Anna Arnoldi,* Marica Carughi, Gandolfina Farina, Lucio Merlini, and Maria G. Parrino

The mechanism of fungicidal action of phytoalexins has not yet been clearly established. Some authors have suggested that they can give rise to radicals upon irradiation with UV light; therefore, we have synthesized some stilbenes, chalcones, and isoflavans structurally related to phytoalexins, modified in order to obtain possible free-radical intermediates of different stability (either radical scavengers or chain propagating). They were tested in vitro and in vivo against some phytopathogenic fungi. It seems that steric hindrance due to *tert*-butyl groups has a negative effect, while allyl chains give rise to a compound with the widest spectrum of activity. Some commercial antioxidants were also tested; they have specific activity against some pathogens.

Phytoalexins are low molecular weight chemicals that plants produce and accumulate in response to infection especially of fungal origin (Deverall, 1982).

Many studies have been done on the role of phytoalexins in the host–pathogen relationship, with the final purpose of using the natural response of plants against infection instead of the traditional treatments with fungicides. However, in comparison with modern synthetic fungicides, phytoalexins are poorly active in vitro ($ED_{50} \sim 10^{-4}$, 10^{-5} M) and almost inactive in in vivo tests (Rathmell and Smith, 1980). We have, therefore, undertaken a program of structural modification of some phytoalexins from Papilionaceae with the aim of obtaining more active compounds (Arnoldi et al., 1986).

It has not yet been clarified satisfactorily why phytoalexins are fungitoxic; most of the results are summarized in the review by (Smith) 1982. Within the group of isoflavonoid phytoalexins, structural and functional damage of membranes has been demonstrated in plant and animal cells. Glyceollin (Kaplan et al., 1980) and phaseollin (Skipp et al., 1977) inhibit oxygen uptake by mitochondria from, respectively, soybean and table beet.

The formation of phytoalexins is generally accompanied by lignification, a process in which glucose 6-phosphate dehydrogenase and peroxidase are quite active and large amounts of free radicals are formed.

Upon irradiation with ultraviolet light some isoflavonoid phytoalexins inactivate glucose 6-phosphate dehydrogenase in vitro, a process in which formation of free radicals was suggested to be the possible cause (Bakker et al., 1983). Moreover, free radicals could damage membranes by favoring the peroxidative degradation of polyunsaturated fatty acids.

In recent years great attention has been paid to the possible oxidative damage inflicted by reactive oxygen species to a wide variety of compounds (DNA, proteins, carbohydrates, lipids) (Sies, 1986). Different biological processes such as inflammation, carcinogenesis, aging, and radiation appear to involve these damages. Enzymatic and nonenzymatic antioxidants were tested in biological systems, for example against inflammation (Bonta et al., 1980).

As we have observed that many natural or synthetic antioxidants are phenols and are structurally related to phytoalexins, we have modified some phytoalexins in order to increase their possible antioxidant properties: In particular we have introduced *tert*-butyl substituents ortho to the phenol group in order to obtain compounds that can give very stable phenoxy radicals which cannot give radical chains.

We have synthesized some analogues of resveratrol, a stilbene phytoalexin from *Arachis hypogea* (Ingham, 1976), obtaining compounds **1–3** (Figure 1). Some other natural (Gorham, 1980) or synthetic stilbenes (Inamori et al., 1984) have fungicidal activity.

We have also prepared chalcones **4–8**. Chalcones are biosynthetically related to phytoalexins, although no stress compound has been reported in this class. Some of them have antifungal activity (Dimmock and Wong, 1976), while others are antioxidants (Dziedzic and Hudson, 1983).

Compound **9** has the isoflavan structure present in some of the phytoalexins with the highest antifungal activity such as (–)-phaseollinisoflavan (Van Etten, 1976). Compounds **10** and **11** are structurally related to **9**. The commercial antioxidants **12**, **14–16** (Figure 2), and a structural analogue of **12**, compound **13**, were also tested. They all are phenols, and compound **14** in particular has the benzopyran nucleus present in many phytoalexins.

In a second part of the research, we prepared two simple stilbenes, **17** and **18**, and a butadiene, **19** (Figure 3), which could react easily with oxygen, generating very reactive free radicals owing to their structure (delocalized radicals with many different reactive positions).

All the compounds were tested in vivo and in vitro against phytopathogenic fungi.

MATERIALS AND METHODS

Chemistry. Melting points are uncorrected. ^1H NMR spectra were determined on a Varian EM-390 spectrometer at 90 MHz or on a Bruker WP 80SY at 80 MHz with TMS as internal standard and are expressed in δ. Mass spectra were recorded on a Finnigan 4021 instrument equipped with super INCOS data system.

Column flash chromatography was performed on silica gel Merck SI-60 (230–400 mesh).

Anhydrous THF was distilled immediately before use from sodium/benzophenone. Acetone and 2-butanone were distilled from K_2CO_3. Anhydrous toluene was distilled from P_2O_5.

Compound **1** was obtained by reaction of phenylacetic acid, piperidine, and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in xylene (Starnes et al., 1969). Compound **2** was obtained by reacting 4-methoxyphenylacetic acid, piperidine, 4-(dimethylamino)pyridine, and 3,5-di-*tert*-butyl-

Dipartimento di Scienze Molecolari Agroalimentari, Sezione di Chimica (A.A., M.C., L.M.), and Istituto di Patologia Vegetale (G.F., M.G.P.), Facoltà di Agraria, Università di Milano, Via Celoria 2, I-20133 Milano, Italy.

0021-8561/89/1437-0508$01.50/0

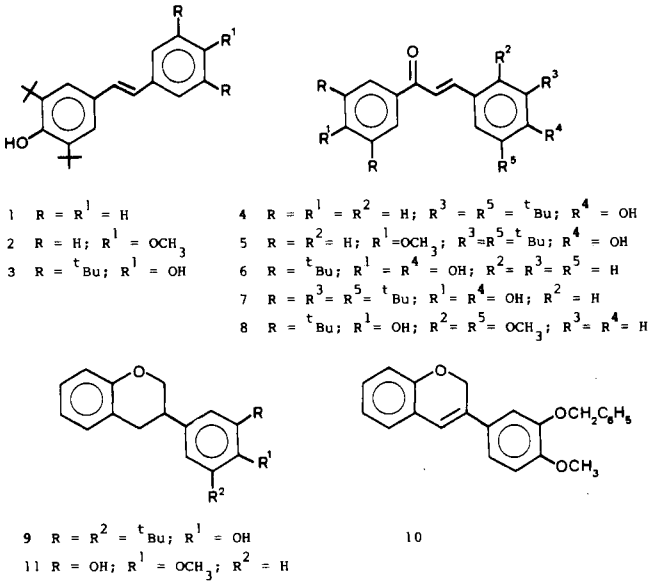


Figure 1. Structures of compounds 1-11.

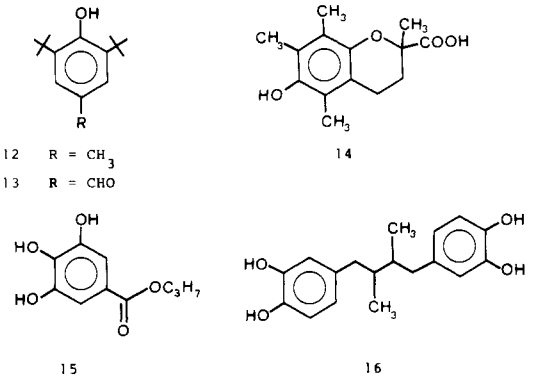


Figure 2. Structures of some known antioxidants.

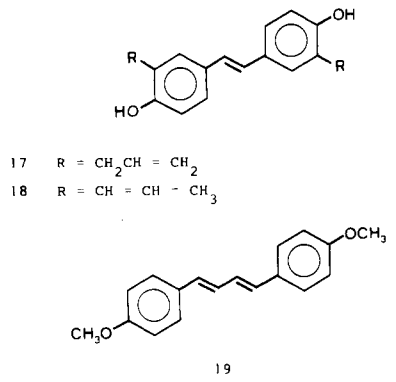


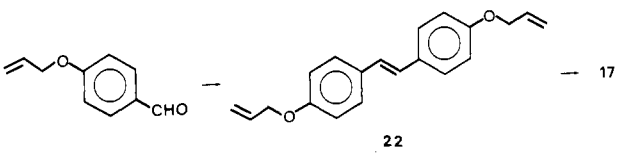
Figure 3. Structures of stilbene derivatives 17–19.

4-hydroxybenzalehyde (Cox et al., 1978). Compound **3** was obtained following a procedure of Baloch et al. (1971): Oxidation of 2,6-di-*tert*-butyl-4-methylphenol with Ag_2CO_3 on Celite gave a stilbene quinone that was then reduced with Zn dust and acetic acid to 4,4′-dihydroxystilbene (**3**).

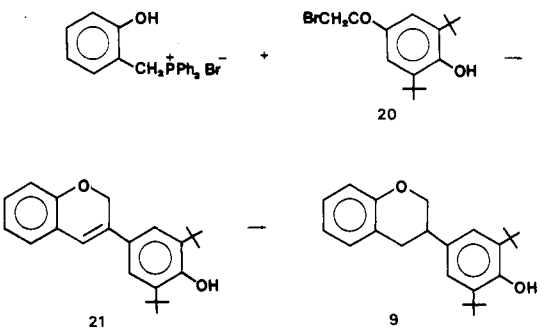
Synthesis of New Compounds. The usual synthesis of chalcones in basic conditions, generally NaOH or KOH in ethanol, was applied only to compound **8**. Acidic conditions (HCl in methanol) were preferred for compounds **4–7**; in fact the salification of the phenolic group in 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde or acetophenone decreases strongly their reactivity in basic conditions (Adams, 1967).

Condensation of [(2-hydroxyphenyl)methyl]triphenylphosphonium bromide (Begasse and Le Corre, 1980) with

Scheme I



Scheme II



the α-bromo ketone ***20*** produced isoflavene **21**. The low yield is probably due to the low electrophilicity of the keto group of **20** in basic medium (Scheme I). α-Bromo ketone **20** was obtained by bromination with $CuBr_2$; in our hands the literature procedure (Fujii et al., 1978) gave a mixture of starting material and of monobromo and dibromo derivatives. Compound **21** was then reduced with H_2 in the presence of a Pd catalyst to give the isoflavan **9**.

With a similar procedure, compounds **10** and **11** were obtained; in this case yields were more satisfying. Compounds **17** and **18** were obtained with the procedure shown in Scheme II. Reductive dimerization of 4-(allyloxy)-benzaldehyde with $TiCl_2$ in THF (McMurry and Fleming, 1974; Lenoir, 1977) gave stilbene **22**, which was submitted to a double Claisen rearrangement by heating at 200 °C to give compound **17**. The allyl groups were rearranged to propenyl with bis(benzonitrile)palladium chloride in toluene (Daviesand and Di Michiel, 1973).

*Compound **4***. 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde (1.17 g, 5 mmol), acetophenone (0.6 g, 5 mmol), and methanol (75 mL) saturated with HCl were stirred overnight at room temperature. The mixture was poured in ice and extracted with ethyl ether (3 × 30 mL). The organic layer was washed with water, dried, and concentrated to 10 mL. Compound **4** crystallized from the solution: 1.29 g (76% yield); mp 180–182 °C (Katsumi et al., 1986); NMR ($CDCl_3$) δ 1.48 (s, 18 H, CH_3), 5.58 (OH), 7.3 (1 H, d, J = 14, H-2), 7.3–7.6 (5 H), 7.6 (1 H, d, J = 14, H-3), 7.85–8.1 (2 H); MS, m/z (%) 336 (66), 321 (56), 279 (11), 265 (11), 191 (3), 218 (5), 115 (7), 105 (100), 91 (9), 77 (63), 57 (41); IR (cm^{-1}) 3500, 1650, 1380, 1100. Anal. Calcd for $C_{23}H_{28}O_2$: C, 82.10; H, 8.39. Found: C, 81.96; H, 8.39.

*Compound **5***. It was obtained in 41.2% yield with a similar procedure from 4-methoxyacetophenone: mp 146–147 °C; NMR ($CDCl_3$) δ 1.48 (18 H, s, CH_3), 3.88 (3 H, s, OCH_3), 5.52 (OH), 6.95 (2 H, d, J = 9, H-3′, H-5′), 7.2–7.8 (4 H), 7.98 (2 H, d, J = 9, H-2′, H-6′); MS, m/z (%) 366 (100), 351 (55), 309 (14), 295 (15), 191 (7), 165 (3), 135 (50), 57 (17). Anal. Calcd for $C_{24}H_{30}O_3$: C, 79.09; H, 7.74. Found: C, 78.93; H, 7.81.

*Compound **6***. It was obtained in 10% yield with a similar procedure from 4-hydroxybenzaldehyde (720 mg, 5.9 mmol) and 3,5-di-*tert*-butyl-4-hydroxyacetophenone (Hiroshi et al., 1982): 1.46 g (5.9 mmol); mp 168–170 °C; NMR ($CDCl_3$) δ 1.49 (18 H, s, CH_3), 5.73 (OH), 6.4 (OH), 6.9 (2 H, d, J = 9, H-3″, H-5″), 7.14 (1 H, d, J = 17, H-2), 7.53 (2 H, d, J = 9, H-2″, H-6″), 7.58 (1 H, d, J = 17, H-3),

7.91 (2 H, s, H-2′, H-6′); MS, m/z (%) 352 (30), 337 (43), 324 (10), 309 (4), 295 (17), 233 (16), 198 (14), 154 (15), 147 (100), 119 (30), 107 (14). Anal. Calcd for $C_{23}H_{28}O_3$: C, 78.37; H, 8.01. Found: C, 78.25, H, 8.09.

Compound 7. It was obtained in 36% yield with a similar procedure from 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (560 mg, 2.4 mmol) and 3,5-di-*tert*-butyl-4-hydroxyacetophenone (595 mg, 2.4 mmol): mp 60–61 °C; NMR ($CDCl_3$) δ 1.47 (36 H, s, CH_3), 5.51 and 5.68 (OH), 7.35 (1 H, d, J = 15, H-2), 7.48 (2 H, s, H-2″, H-6″), 7.78 (1 H, d, J = 15, H-3), 7.9 (2 H, s, H-2′, H-6′); MS, m/z (%) 464 (6), 449 (5), 251 (9), 248 (21), 233 (100), 205 (24), 173 (10), 149 (23), 143 (14). Anal. Calcd for $C_{31}H_{44}O_3$: C, 80.13; H, 9.55. Found: C, 79.92; H, 9.62.

Compound 8. A mixture of 2,5-dimethoxybenzaldehyde (1.0 g, 6.04 mmol), 3,5-di-*tert*-butyl-4-hydroxyacetophenone (1.5 g, 6.04 mmol), ethanol (3 mL), and 1.6 M NaOH in water (6 mL) was heated at 70 °C for 2 h with stirring. The mixture was diluted with water and acidified with HCl. The solid was filtered and washed with methanol. The organic solvents were evaporated in part; the mixture was extracted with ethyl acetate (3 × 20 mL). The residue obtained after concentration of the solvent was purified by column chromatography: 17% yield; mp 79–81 °C (hexane–ethyl ether); NMR ($CDCl_3$) δ 1.5 (18 H, s, CH_3), 3.81 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 5.69 (OH), 6.88 (2 H, m), 7.15 (1 H, m, H-6″), 7.54 (1 H, d, J = 16, H-2), 7.9 (2 H, s, H-2′, H-6′), 8.00 (1 H, d, J = 16, H-3); MS, m/z (%) 396 (9), 365 (100), 349 (2), 191 (11), 176 (14). Anal. Calcd for $C_{25}H_{32}O_4$: C, 75.72; H, 8.13. Found: C, 75.39; H, 7.95.

Compound 9. 1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-ethanone (5.8 g, 23 mmol) in anhydrous ethyl acetate (140 mL) was added dropwise into a slurry of $CuBr_2$ (10.4 g, 46 mmol) in chloroform (40 mL) at reflux. The mixture was stirred for 3 h and filtered; the solvent was evaporated. The crude α-bromo ketone **20** was crystallized from hexane: 5.5 g (73% yield); mp 103 °C [lit. mp 95–97 °C (Volod'kin et al., 1967)].

Sodium methoxide (2.47 N in methanol, 1.4 mL) was added dropwise in a slurry of [(2-hydroxyphenyl)-methyl]triphenylphosphonium bromide (Begasse and Le Corre, 1980) (1.35 g, 3 mmol) in toluene (20 mL) with vigorous stirring. After 20 min α-bromo ketone **20** (1 g, 3 mmol) was added, and after 30 min, sodium methoxide (2.47 N in methanol, 2.6 mL); the mixture was heated 5 h. HCl was added (1 M, 10 mL), and the mixture was extracted with ethyl acetate (2 × 20 mL). After concentration of the solvent, the residue was purified by column chromatography. 3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2*H*-1-benzopyran (**21**) was obtained: 47% yield; mp 90–91 °C (methanol); NMR ($CDCl_3$) δ 1.45 (18 H, s, CH_3), 5.10 (2 H, AB, H-2), 5.25 (OH), 6.60 (1 H, H-3), 6.7–7.2 (6 H).

This compound (300 mg, 0.89 mmol) was hydrogenated in ethyl acetate (15 mL) in the presence of 10% Pd on carbon (80 mg). The catalyst was removed, and the mixture was filtered on a 2-cm bed of silica gel and concentrated. Compound **9** was obtained in 66% yield: mp 116 °C; NMR ($CDCl_3$) δ 1.55 (18 H, s, CH_3), 3.15 (3 H, m, H-3 and H-4), 4.2 (2 H, m, H-2), 5.20 (OH), 6.9–7.2 (6 H). Anal. Calcd for $C_{23}H_{30}O_2$: C, 77.93; H, 8.53. Found: C, 77.84; H, 8.76.

Compound 10. [(2-Hydroxyphenyl)methyl]triphenylphosphonium bromide (8 g, 17 mmol) (Begasse and Le Corre, 1980) in anhydrous toluene (80 mL) was added with 2.47 N sodium methoxide in methanol (2 mL) under nitrogen and with vigorous stirring. After 10 min, 1-[3-(benzyloxy)-4-methoxyphenyl]-2-bromoethanone (6 g, 17 mmol) (Fujii et al., 1978) was added. The yellow mixture was heated at reflux, and 2.47 N sodium methoxide in methanol (2 mL) was added dropwise over 30 min. The reaction was heated for 5 h, the inorganic salts were filtered, the toluene was evaporated, and the residue was purified by column chromatography to give **10**: 3.5 g (60% yield); mp 102–103 °C (hexane); NMR ($CDCl_3$) δ 3.87 (3 H, s, OCH_3), 5.02 (2 H, d, J = 1, H-2), 5.17 (2 H, s, OCH_2Ph), 6.62 (1 H, s, H-4), 6.8–7.5 (7 H). Anal. Calcd for $C_{23}H_{20}O_3$: C, 80.23; H, 5.81. Found: C, 80.12; H, 5.80.

Compound 11. Compound **10** (1 g, 2.9 mmol) was hydrogenated with 10% Pd/C in ethyl acetate (20 mL). The catalyst was filtered, the solvent was evaporated, and the solid residue was purified by column chromatography: 500 mg (67% yield); mp 95–96 °C (cyclohexane); NMR ($CDCl_3$) δ 3.0 (3 H, m, H-3 and 4), 3.87 (3 H, s, OCH_3), 3.9–4.5 (2 H, m, H-2), 5.6 (OH), 6.7–7.3 (7 H, aromatic). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.99; H, 6.31.

Compound 17. Under vigorous stirring $TiCl_4$ (2.5 mL, 22.2 mmol) was added dropwise in anhydrous THF (60 mL), and a yellow precipitate formed. Zn powder (2.9 g, 65.4 mmol) was added, and after 5 min pyridine (1.5 mL) and 4-(allyloxy)benzaldehyde (3 g, 18.5 mmol) in THF (28 mL) were added dropwise. The brown mixture was refluxed for 14 h and then poured in HCl and ice and extracted with dichloromethane (3 × 20 mL). The organic layer was dried and concentrated. The solid residue was crystallized from ethanol, to give 4,4′-bis(allyloxy)stilbene (**22**): 1.88 g (70%); mp 190–191 °C; NMR ($CDCl_3$) δ 4.55 (4 H, m, OCH_2), 5.34 (4 H, m, $=CH_2$), 6.05 (2 H, m, CH=), 6.9 (4 H, d, J = 8), 6.9 (2 H, s, CH), 7.4 (4 H, d). Stilbene **22** (1.88 g, 6.16 mmol) was heated at 200 °C for 7 h in *N*,*N*-diethylaniline (24 mL) under nitrogen. The mixture was poured in 7 M H_2SO_4 (30 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with water, dried, and concentrated. The crude residue was crystallized from cyclohexane: 940 mg (50%); mp 128 °C; NMR ($CDCl_3$) δ 3.43 (4 H, d, J = 6, CH_2), 4.95 (20 H), 5.15 (4 H, m, CH_2=), 6.0 (2 H, m, CH=), 6.6–7.4 (8 H). Anal. Calcd for $C_{20}H_{20}O_2$: C, 82.15; H, 6.89. Found: C, 82.26; H, 6.85.

Compound 18. 3,3′-Diallyl-4,4′-dihydroxystilbene (**17**) (940 mg, 3.2 mmol) and bis(benzonitrile)palladium dichloride (100 mg) were stirred for 17 h in anhydrous toluene (5 mL). The mixture was filtered, and the solid was washed repeatedly with chloroform. The organic solvent was evaporated, and the residue was crystallized from toluene: 190 mg (20% yield); mp 197–199 °C; NMR ($CDCl_3$) δ 1.9 (6 H, d, J = 6, CH_3), 2.3 (2 OH), 6.3 (2 H, dq, J = 16 and 6, =C*H*Me), 6.75 (2 H, d, J = 16, =CHAr), 6.8 (2 H, d, J = 8, H-5 and H-5′), 6.9 (2 H, s, CH), 7.2 (2 H, dd, J = 8 and 3, H-6, H-6′), 7.5 (2 H, d, J = 3, H-2, H-2′). Anal. Calcd for $C_{20}H_{20}O_2$: C, 82.15; H, 6.89. Found: C, 82.15; H, 6.91.

Biological Assays. The 19 compounds were tested in vitro and in vivo against different phytopathogenic fungi.

Test Fungi. Pathogenic strains of the following fungi were used: *Botrytis cinerea* Pers. on malt agar, *Colletotrichum lindemuthianum* Sacc. et Magn. on neopeptone yeast extract glucose agar, *Phytophtora infestans* (Mont.) De Bary on V8 juice agar. *Sphaerotheca fuliginea* (Sch.) Solmon and *Uromyces appendiculatus* (Pers.) Link were maintained on stock plants.

Test for Fungitoxicity in Vitro. The compounds were assayed against mycelial growth by the commonly used poisoned food technique.

Table I. Activity of Compounds Tested

	in vitro tests[a]			in vivo tests[a]			
compd	B[b]	C	P	U/P[c]	S/C	C/P	P/S
1	>100	>100	>100	500	>500	>1000	
2	>100	>100	>100	>500	>500		
3	>100	>100	>100	>500	>500		
4	>100	>100	>100	>500	>500		
5	>100	>100	>100	>500	>500		
6	70	40	>100	>500	>500	>1000	
7	>100	>100	>100	>500	>500		
8	>100	>100	>100	>500	>500		
9				>1000	>500	>500	
10	50	>100	>100	>500	>500	>500	
11	25	>100	>100	>500	>500	>500	
12	30	100	>100	>500	>500	>1000	>500
13	>100	>100	>100	500	>500		
14	>100	>100	38	>500	>500		>500
15	>100	>100	30	>500	>500		170
16	>100	>100	<12.5	>500	>500		>500
17	<12.5	>100	20	200	>1000		400
18	>100	25	>100	>1000	>1000		
19	35	>100	>100	>1000	>1000		

[a] Activity expressed as ED_{50} (mg L^{-1}). [b] Key: B = *B. cinerea*; C = *C. lindemuthianum*; P = *P. infestans*. [c] Key: U/P = *U. appendiculatus* on *P. vulgaris*; S/C = *S. fuliginea* on *C. sativus*; C/P = *C. lindemuthianum* on *P. vulgaris*; P/S = *P. infestans* on *Solanum lycopersicum*.

Solutions of each compound were prepared by dissolving the appropriate amounts of compound in 2 mL of Me_2SO plus Tween 20 (0.01%). Equal volumes of Me_2SO containing diluted compounds were added to sterile cool agar media to give concentrations of 100, 50, 25, and 12.5 mg L^{-1} for each compound. A zero concentration treatment containing the same percentage of Me_2SO and Tween to ensure equivalent concentrations of these components in all the treatments was prepared for each fungus.

Compound-amended agar media were dispersed aseptically into 9-cm-diameter plastic Petri dishes (10 mL/dish). Each dish was inoculated with two mycelial discs (7-mm diameter) cut from the periphery of colonies actively growing. Two replicate dishes were used for each concentration, together with dishes containing toxicant-free media.

The degree of inhibition of growth was calculated from the mean difference between treatment and control as a percentage of the latter. Percentage growth inhibition was plotted on a probit scale against chemical concentrations in order to obtain 50% inhibition concentrations (ED_{50} values).

Tests for Fungitoxicity in Vivo. Tests were performed on the following pathogen–host combinations: *S. fuliginea/Cucumis sativus*, *U. appendiculatus/Phaseolus vulgaris*, and, in some cases, *C. lindemuthianum/P. vulgaris*, *P. infestans/Solanum lycopersicum*.

Plants. Test plants were grown in pots containing sterilized soil and maintained in either greenhouse or growth room (temperature 22–23 °C, RH 75 ± 10%).

Direct Protectant Activity. Compounds were homogenized in a minimal amount of water containing 0.01% Tween 20 and then diluted to concentrations of 1000, 500, and 250 mg L^{-1}. Both surfaces of plant leaves were sprayed to run off and were allowed to dry before being inoculated. Inoculation was performed 24 h after treatment.

The area of inoculated leaves covered by disease symptoms was assessed on a 6-point scale from 0 to 5, in which 0 corresponded to no visible symptoms and 5 to 100% of the leaves covered.

The compound activity was calculated on the basis of the percentage inhibition of the disease in comparison with the inoculated untreated plants. The ED_{50} values were estimated from dosage–response curves as indicated above.

RESULTS

The results for the 19 compounds in the different tests are reported in Table I. Three compounds (**14–16**) have specific activity against *P. infestans*, four (**10–12, 19**) act only against *B. cinerea*, and one (**18**) is selective on *C. lindemuthianum*.

Compound **6** is fungitoxic against two pathogens, *B. cinerea* and *C. lindemuthianum*, while compound **17** has the widest range of activity, being active against three different pathogens (*B. cinerea*, *P. infestans*, *U. appendiculatus*). Only one of them (**17**) shows good activity in the protectant test against *U. appendiculatus* on *P. vulgaris*, and, in practice, none of them is active against cucumber powdery mildew.

In detail, none of the compounds tested has an appreciable antifungal activity, except **16** and **17** that in vitro present an ED_{50} value <12.5 against *P. infestans* and *B. cinerea*, respectively. In the test (*P. infestans/S. lycopersicum*), better control is obtained by compound **15** (ED_{50} = 170). Compound **17** is relatively good in protecting bean plants against rust (ED_{50} = 200).

DISCUSSION

Among the commercial antioxidants tested, BHT (**12**) has specific activity on *B. cinerea* and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (**14**) and nordihydroguajaretic acid (**16**) on *P. infestans*. Propyl gallate (**15**) was active either in vitro or in vivo on *P. infestans*. Slight antifungal activity on nonphytopathogenic fungi has been already reported for the last compound, but only at very high dosage (3000 mg L^{-1}) (Eubanks and Beuchat, 1982; Zeelie and McCarthy, 1983).

As the antioxidants we have tested have very similar antifungal activity, though their structures are rather different, their common antioxidant properties could be related to the mechanism of action. It should be, therefore, interesting to modulate the structure of some natural phytoalexins in order to stabilize possible radical intermediates and to give them antioxidant properties.

However, the introduction of *tert*-butyl substituents at the ortho position in phenolic groups to enhance the antioxidants properties of stilbenoid phytoalexins such as resveratrol has produced compounds completely lacking antifungal activity (**1–3**). On the contrary, the high activity of compound **17** suggests that allylic chains are particularly favorable; in fact, this compound retains activity also in in vivo tests. The apparent slight modification of exchanging allyl with propenyl chains, which did not change the lipophilicity of the molecule, totally depressed the activity. This has not been observed with natural resveratrol derivatives in which 4-(3-methylbut-1-enyl) and 4-(3-methylbut-2-enyl) derivatives have similar activity (Aguamah et al., 1981).

In the class of chalcones (**4–8**), the best compound was **6**, the only one with an OH group free of steric hindrance. Phenolic groups seem to be very important for antifungal activity; the same observation was made in the class of isoflavonoid phytoalexins (Van Etten, 1976) and phenyl-substituted coumarins (Arnoldi et al., 1986). It seems, therefore, that although some antifungal activity can be detected with antioxidant compounds, inhibition of free-radical formation is not directly connected to it and their mechanism of action is due to other not yet clarified features.

ACKNOWLEDGMENT

This work was supported by the Italian National Research Council. Special Grant IPRA, Subproject 1, Paper

No. 1849.

Registry No. **1**, 19566-71-3; **2**, 119038-89-0; **3**, 2950-01-8; **4**, 83677-22-9; **5**, 119038-90-3; **6**, 119038-91-4; **7**, 119038-92-5; **8**, 119038-93-6; **9**, 119038-94-7; **10**, 119038-95-8; **11**, 119038-96-9; **12**, 128-37-0; **13**, 1620-98-0; **14**, 56305-04-5; **15**, 121-79-9; **16**, 500-38-9; **17**, 119038-97-0; **18**, 119038-98-1; **19**, 43212-67-5; **20**, 14386-64-2; **21**, 119038-99-2; **22**, 119073-04-0; *p*-H_2C=$CHCH_2OC_6H_4CHO$, 40663-68-1; *o*-$HOC_6H_4CH_2P^+Ph_3Br^-$, 70340-04-4; phenylacetic acid, 103-82-2; piperidine, 110-89-4; 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, 1620-98-0; 4-methoxyphenylacetic acid, 104-01-8; 4-(dimethylamino)pyridine, 1122-58-3; 2,6-di-*tert*-butyl-4-methylphenol, 128-37-0; acetophenone, 98-86-2; 4-methoxyacetophenone, 100-06-1; 3,5-di-*tert*-butyl-4-hydroxyacetophenone, 14035-33-7; 2,5-dimethoxybenzaldehyde, 93-02-7; 1-[3-(benzyloxy)-4-methoxyphenyl]-2-bromoethanone, 69700-17-0; bis(benzonitrile)palladium dichloride, 14220-64-5.

LITERATURE CITED

Adams, J. H. The Influence of Bulky Substituents on the Synthesis of 4-Hydroxy-3,5-dialkyl Flavanoids. *J. Org. Chem.* **1967**, *32*, 3992.

Aguamah, G. E.; Langcake, P.; Leworthy, D. P.; Page, J. A.; Pryce, R. J.; Strange, R. N. Isolation and Characterization of Novel Stilbene Phytoalexins from *Arachis hypogea*. *Phytochemistry* **1981**, *20*, 1381.

Arnoldi, A.; Farina, G.; Galli, R.; Merlini, L.; Parrino, M. G. Analogues of Phytoalexins. Synthesis of Some 3-Phenylcoumarins and Their Fungicidal Activity. *J. Agric. Food Chem.* **1986**, *34*, 185.

Bakker, J.; Gommers, F. J.; Smits, L.; Fuchs, A.; de Vries, F. W.; Photoactivation of Isoflavonoid Phytoalexins: Involvement of Free Radicals. *Photochem. Photobiol.* **1983**, *38*, 323.

Baloch, V.; Fetizon, M.; Golfier, M. Oxidations with Silver Carbonate/Celite. V. Oxidations of Phenols and Related compounds. *J. Org. Chem.* **1971**, *36*, 1339.

Begasse, B.; Le Corre, M. Nouvelles voies d'Accès aux Benzopyrannes. III. 2H-Benzo(b)pyrannes (chromènes-3). *Tetrahedron* **1980**, *36*, 3409.

Bonta, I. L.; Parnham, M. J.; Vincent, J. E.; Bragt, P. C. Anti-Rheumatic Drugs: Present Deadlock and New Vistas. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier/North-Holland Biomedical Press: Amsterdam, 1980; Vol. 17, p 185.

Cox, M. T.; Jaggers, S. E.; Jones, G. Linked Aryl Aryloxypropanolamines as a New Class of Lipid Catabolic Agents. *J. Med. Chem.* **1978**, *21*, 182.

Daviesand, N. R.; Di Michiel, A. D. The Homogeneous Catalyzed Isomerization of 2-Allylphenol and of 2-Propenylphenol in the Presence of Palladium Catalyst. *Aust. J. Chem.* **1973**, *26*, 1529.

Deverall, B. J. Introduction. *Phytoalexins*; Bailey, J. A., Mansfield, J. W., Eds.; Blackie: Glasgow and London, 1982; p 1.

Dimmock, Y. R.; Wong, L. C. M. Bioactivities and potential uses in drug design of acyclic α,β-unsaturated ketones. *Can. J. Pharm. Sci.* **1976**, *11*, 35.

Dziedzic, S. Z.; Hudson, B. J. F. Polyhydroxy Chalcones and Flavanones as Antioxidants for Edible Oils. *Food Chem.* **1983**, *12*, 205.

Eubanks, V. L.; Beuchat, L. R. Effects of Antioxidants on Growth, Sporulation and Pseudomycelium Production by *Saccharomyces cerevisiae*. *J. Food Sci.* **1982**, *47*, 1717.

Fujii, T.; Yoshifujii, S.; Ohba, M. Preparation of some Ring-oxygenated Phenacyl Bromides. *Chem. Pharm. Bull.* **1978**, *26*, 3218.

Gorham, J. The Stilbenoids. *Prog. Phytochem.* **1980**, *6*, 203.

Hiroshi, S.; Shigejoshi, K.; Masatoshi, M. Acylation of Sterically Hindered Phenols in the Presence of Anhydrous Aluminum Chloride. *Kanazawa Daigaku Kogakibu Kijo* **1982**, *15*, 71; *Chem. Abstr.* **1982**, *97*, 197910d.

Inamori, Y.; Kubo, M.; Kato, Y.; Yasuda, M.; Baba, K.; Kozawa, M. The Antifungal Activity of Stilbene Derivatives. *Chem. Pharm. Bull.* **1984**, *32*, 801.

Ingham, J. L. 3,5,4'-Trihydroxystilbene as a Phytoalexin from groundnuts (*Arachis hypogea*). *Phytochemistry* **1976**, *15*, 1791.

Kaplan, D. T.; Keen, N. T.; Thomanson, I. J. Studies on the Mode of Action of Glyceollin in Soybean. Incompatibility to the Root Knot. *Physiol. Plant Pathol.* **1980**, *16*, 319.

Katsumi, I.; Kondo, H.; Fuse, Y.; Yamashita, K.; Hidaka, T.; Hosoe, K.; Takeo, K.; Yamashita, T.; Watanabe, K. Studies on styrene derivatives. II. Synthesis and antiinflammatory activity of 3,5-di-tert-butyl-4-hydroxystyrenes. *Chem. Pharm. Bull.* **1986**, *34*(4), 1619–1627.

Lenoir, D. Synthese tetrasubstituierter Ethylene durch Reduktive Kupplung von Ketone mittles Titan (II)-salzen. Zur Anwendung der Methode. *Synthesis* **1977**, 553.

McMurry, J. E.; Fleming, M. P. A New Method for the Reductive coupling of Carbonyls to Olefins. Synthesis of β-Carotene. *J. Am. Chem. Soc.* **1974**, *96*, 4708.

Rathmell, W. G.; Smith, D. A. Lack of Activity of Selected Isoflavonoid Phytoalexins as Protectant Fungicides. *Pestic. Sci.* **1980**, *11*, 568.

Sies, H. Biochemistry of Oxidative Stress. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1058.

Skipp, R. A.; Selby, C.; Bailey, J. A. Toxic effects of Phaseollin on Plant Cells. *Physiol. Plant Pathol.* **1977**, *10*, 221.

Smith, D. A. Toxicity of Phytoalexins. *Phytoalexins*; Bailey, J. A., Mansfield, J. W., Eds.; Blackie: Glasgow and London, 1982; p 218.

Starnes, W. H.; Myers, J. A.; Lauff, J. J. Reaction of a Quinone Methide with Triethyl Phosphite. *J. Org. Chem.* **1969**, *34*, 3404.

Van Etten, H. D. Antifungal Activity of Pterocarpans and Other Selected Isoflavonoids. *Phytochemistry* **1976**, *15*, 655.

Volod'kin, A. A.; Portnykh, N. V.; Ershov, V. V. 2-Bromoalkyl-4-hydroxy-3,5-di-*tert*-butylphenyl ketones. *Izv. Akad. Nauk. SSSR, Ser. Khim.* **1967**, 947; *Chem. Abstr.* **1968**, *68*, 39279b.

Zeelie, J. J.; McCarthy; T. J. The Antimicrobial Activity of Phenolic Antioxidants in Aqueous Solutions. *Cosmet. Toiletries* **1982**, *97*, 61; *Chem. Abstr.* **1983**, *98*, 31348x.

Received for review April 26, 1988. Accepted September 8, 1988.