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REGULATION OF ENZYMIC OXIDATION OF INDOLE-3-ACETIC ACID BY PHENOLS: STRUCTURE–ACTIVITY RELATIONSHIPS

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Key Word Index—Phenols; indole-3-acetic acid; IAA-oxidase; peroxidase.

Abstract—Mono- and diphenols were tested for their effects on the decarboxylation of [1-^{14}C]IAA catalysed by purified horseradish peroxidase (EC 1.11.1.7) in the presence or absence of 2,4-dichlorophenol (DCP). The number of hydroxyl groups and their position relative to each other and the nature and position of other substituents on the aromatic ring were found to affect the activity. Although the effects were complex, the following generalizations may be made. (1) Monophenols produce activation when no other cofactor is present. *p*-Substituted monophenols are more active than *o*- or *m*-compounds. In the presence of DCP, the activity varies from slight activation to strong inhibition. (2) *m*-Diphenols also produce activation in the absence of other cofactors while *o*- and *p*-diphenols, with the exception of 3,4-dihydroxyacetophenone and 3,4-dihydroxypropiophenone, produce strong inhibition in the presence or absence of DCP. The *o*-diphenols are degraded in the IAA-oxidizing enzyme system and thus produce only a temporary inhibition. (3) *m*-Diphenols and 3,4-dihydroxyacetophenone produce a sustained inhibition in the presence of DCP. (4) Substitution at position 2 significantly alters the activity of *m*-diphenols. (5) *O*-Methylation alters the activity of most *o*-diphenols. In the absence of DCP, *o*-methoxyphenols and certain other phenols such as 3,4-dihydroxyacetophenone and 2,6-dihydroxyacetophenone either promote or inhibit IAA oxidation depending on concentration.

INTRODUCTION

Many natural phenols affect the rate of enzymic oxidation of indole - 3 - acetic acid (IAA) *in vitro*. It is generally accepted that *p*-substituted monophenols promote and *o*-diphenols inhibit this reaction. As with the simple phenols, flavonoids with one hydroxyl in the 4'-position stimulated the activity of IAA-oxidase while those with *o*-dihydroxyls in either the A or B ring were inhibitory [1, 2]. More recently, however, an exception to this rule has been observed; 3,4-dihydroxyacetophenone and 3,4-dihydroxypropiophenone were found to be cofactors of IAA oxidation [3]. This finding prompted a re-examination of the relationship between the structure of phenols and their effects on IAA oxidation.

RESULTS AND DISCUSSION

Monophenols

Results from tests with the hydroxybenzoic acids **1**, **2**, **3**, hydroxyacetophenones **4**, **5**, **6** and hydroxyphenylacetic acids **8**, **9**, **10** confirmed that compounds with the hydroxyl group in the *p*-position had the highest cofactor activity in the horseradish peroxidase (HRP)-catalysed decarboxylation of IAA (Table 1). Significantly lower activity was observed for the *m*-hydroxy substituted compounds while the *o*-hydroxy compounds had still lower activity. It has been known that *p*-coumaric and *p*-hydroxybenzoic acids have a higher cofactor activity in IAA oxidation than the corresponding *o*- and *m*-derivatives [4, 5]. Among the monophenols tested, *p*-hydroxyacetophenone had the highest cofactor activity. The activity of 2,2'-dihydroxybenzophenone (**7**) was similar to that of *o*-hydroxyacetophenone.

In the presence of 2,4-dichlorophenol (DCP), a strong cofactor of IAA-oxidase, the hydroxyphenylacetic acids, especially **8** and **10**, and *p*-coumaric acid were highly inhibitory while the hydroxybenzoic acids and hydroxyacetophenones were not. Thus the structure–activity relationship observed for the monophenols in the absence of DCP no longer held when DCP was present.

Diphenols

A comparison of the activity of three simple dephenols, catechol (**12**), resorcinol (**39**) and hydroquinone (**34**), clearly shows differences in their effects on IAA oxidation (Table 1). In the absence of DCP, resorcinol promoted IAA oxidation whereas catechol and hydroquinone inhibited it. Similar results were observed with methyl substituted diphenols, dihydroxybenzoic acids and dihydroxyacetophenones with the exception of compound **24**. In general, when tested at 10^{-4} M concentration, diphenols with the hydroxyl groups in a 1,2- or 1,4-relationship on the aromatic ring were inhibitors whereas those with the hydrolysis in a 1,3-relationship were cofactors. In the presence of DCP, the two groups of diphenols all inhibited IAA oxidation but

Table 1. Relative activity of phenolic compounds in the horseradish peroxidase-catalysed decarboxylation of [1-^{14}C]IAA in the presence or absence of 2,4-dichlorophenol

No.	Compound	% Activation		% Inhibition	
		+DCP	−DCP	+DCP	−DCP
	Monophenols				
1	Salicylic acid	10	27		
2	*m*-Hydroxybenzoic acid	1	26		
3	*p*-Hydroxybenzoic acid		571	7	
4	*o*-Hydroxyacetophenone		17	4	
5	*m*-Hydroxyacetophenone		75	2	
6	*p*-Hydroxyacetophenone	2	792		
7	2,2'-Dihydroxybenzophenone		51	5	
8	*o*-Hydroxyphenylacetic acid		2	52	
9	*m*-Hydroxyphenylacetic acid		38	9	
10	*p*-Hydroxyphenylacetic acid		621	63	
11	*p*-Coumaric acid		49	94	
	o-Diphenols and derivatives				
12	Catechol			100	99
13	Guaiacol			100	99
13	Guaiacol (10^{-6} M)		124		
14	3-Methylcatechol			100	100
15	2,3-Dihydroxybenzoic acid			98	99
16	2-Hydroxy-3-methoxyallylbenzene			100	98
17	4-Methylcatechol			100	99
18	3,4-Dihydroxybenzonitrile			100	99
19	3,4-Dihydroxybenzaldehyde			100	98
20	Vanillin		574	75	
21	3,4-Dihydroxybenzoic acid			100	99
22	4-Hydroxy-3-methoxybenzoic acid			98	69
22	4-Hydroxy-3-methoxybenzoic acid (10^{-5} M)		266		
23	Methyl 3,4-dihydroxybenzoate			100	100
24	3,4-Dihydroxyacetophenone		380	81	
25	4-Hydroxy-3-methoxyacetophenone		375	81	
25	4-Hydroxy-3-methoxyacetophenone (10^{-5} M)		1467		
26	3,4-Dihydroxy-ω-chloroacetophenone			100	99
27	4-Hydroxy-3-methoxybenzyl alcohol			100	100
27	4-Hydroxy-3-methoxybenzyl alcohol (10^{-7} M)		14		
28	3,4-Dihydroxyphenylacetic acid			100	100
29	4-Hydroxy-3-methoxyphenylacetic acid			100	99
29	4-Hydroxy-3-methoxyphenylacetic acid (10^{-7} M)				8
30	3,4-Dihydroxypropiophenone		60	78	
31	Caffeic acid			100	100
32	Ferulic acid			100	100
32	Ferulic acid (10^{-7} M)		40		
33	3-Hydroxy-4-methoxycinnamic acid			99	68
33	3-Hydroxy-4-methoxycinnamic acid (10^{-7} M)		2		
	p-Diphenols and derivatives				
34	Hydroquinone			100	100
35	*p*-Methoxyphenol			100	98
36	2,5-Dihydroxybenzoic acid			99	99
37	2,5-Dihydroxyacetophenone			100	98
38	2-Hydroxy-5-methoxyacetophenone			85	42
	m-Diphenols and derivatives				
39	Resorcinol		2789	34	
40	*m*-Methoxyphenol		1541	34	
41	5-Methylresorcinol		1322	44	
42	3,5-Dihydroxybenzoic acid		103	10	
43	2,4-Dihydroxyacetophenone		1109	11	
44	2-Hydroxy-4-methoxyacetophenone		2	5	
45	2,4-Dihydroxybenzoic acid		679	0	

Table 1—*continued*

No.	Compound	% Activation +DCP	% Activation −DCP	% Inhibition +DCP	% Inhibition −DCP
46	2,4-Dihydroxybenzophenone		403	7	
47	5-Acetyl-2,4-dihydroxyacetophenone	2	12		
48	2-Methylresorcinol		188	95	
49	2,6-Dihydroxyacetophenone		476	73	
50	2-Hydroxy-6-methoxyacetophenone		6	7	
51	2-Hydroxy-6-benzyloxyacetophenone	5	31		
52	2,6-Dihydroxybenzoic acid		23	8	
53	Methyl 2,6-dihydroxybenzoate			51	5
54	2,6-Dihydroxybenzophenone			76	15
55	2,6-Dihydroxyacetophenone oxime			63	22
56	3-Bromo-2,6-dihydroxyacetophenone			100	93
	Triphenols and derivatives				
57	2,3,4-Trihydroxyacetophenone			100	100
58	2,3-Dihydroxy-4-methoxyacetophenone			100	98
59	2,4,6-Trihydroxyacetophenone			99	88
60	2,6-Dihydroxy-4-methoxyacetophenone		122	42	
61	2-Hydroxy-4,6-dimethoxyacetophenone	18	32		

The reaction mixture (2.0 ml) has the following composition: IAA (0.2 mM containing 0.44 μM [1-^{14}C]IAA), K-Pi buffer (50 mM, pH 6), test compound (0.1 mM unless otherwise specified) and HRP (2.5 μg). The phenols were also tested in the presence of 0.1 mM DCP. The average counts of $^{14}CO_2$ collected from the control were 55046 dpm with DCP and 1878 dpm without DCP.

the kinetics of inhibition were different. The similarities and differences are discussed below.

o-Diphenols and derivatives. Of the 13 substituted *o*-diphenols tested, 11 were inhibitors of IAA oxidation either in the presence or absence of DCP. There was little difference in activity between 2,3- and 3,4-diphenols. Several compounds from this group exhibited a temporary type of inhibition, after which IAA oxidation resumed at the same rate as the control [3, 6–8]. TLC indicated that the *o*-diphenols were oxidized in the enzyme system. The oxidation was accelerated when the *o*-diphenols were added a few seconds after IAA, suggesting that enzyme intermediates formed during reaction with IAA were involved in the breakdown of these compounds [9]. Thus, IAA played a significant role in the peroxidase-catalyzed oxidation of *o*-diphenols. Presumably other suitable substrates of peroxidase affect the oxidation of *o*-diphenols similarly. Gelinas [10] has reported degradation of ferulic acid in an IAA-oxidizing system and has proposed that HRP-I and HRP-II were the enzyme forms responsible for the breakdown. Oxidation of *o*-diphenolic inhibitors in the IAA-oxidizing enzyme system explains the temporary type of inhibition produced by these compounds.

3,4-Dihydroxyacetophenone (**24**) and 3,4-dihydroxypropiophenone (**30**) are exceptions to the rule that *o*-diphenols are inhibitors of IAA oxidation (Table 1). Although compound **24** persistently inhibited IAA oxidation in the presence of DCP, it either promoted or inhibited the reaction depending on concentration in the absence of DCP [3]. Lengthening the side-chain of compound **24** decreased the cofactor activity and introducing a chlorine into the terminal methyl group (compound **26**) changed the activity from activation to inhibition. The unique activity of compound **24** compared with that of other *o*-diphenols has been reported [3]. 3,4-Dihydroxybenzaldehyde (**19**) behaved like an ordinary *o*-diphenol and was inhibitory to IAA oxidation over a broad concentration range (10^{-7}–10^{-4} M). The activity of 3,4-dihydroxybenzonitrile (**18**) was also similar to other *o*-diphenols. Duckworth and Coleman [11] have reported that the K_m for oxidation of catechols with substituents in position 4 by mushroom tyrosinase decreased in the following order: H > COMe > CHO > CN, which was the reverse of the electron-withdrawing ability of the substituents. No such relationship was observed for compounds **12**, **24**, **19** and **18** in the horseradish peroxidase-catalysed oxidation of IAA. Thus, the unique activity of 3,4-dihydroxyacetophenone (**24**) is probably unrelated to the inductive effect of the acetyl substituent.

O-Methylation altered the activity of most *o*-diphenols tested. At 10^{-4} M concentration, vanillin (**20**) exhibited cofactor activity in the absence of DCP but other *o*-methoxy phenols were inhibitory (Table 1). At lower concentrations (10^{-7}–10^{-5} M), however, compounds **13**, **22**, **27** and **32** also showed cofactor activities. A comparison of activity of an *o*-methoxy phenol and an *o*-diphenol is shown in Fig. 1. Evidently, 4 - hydroxy - 3 - methoxybenzoic acid (**22**) and certain other *o*-methoxyphenols can either promote or inhibit IAA oxidation depending on concentration used. This behavior is similar to that observed for compound **24**. However, there were

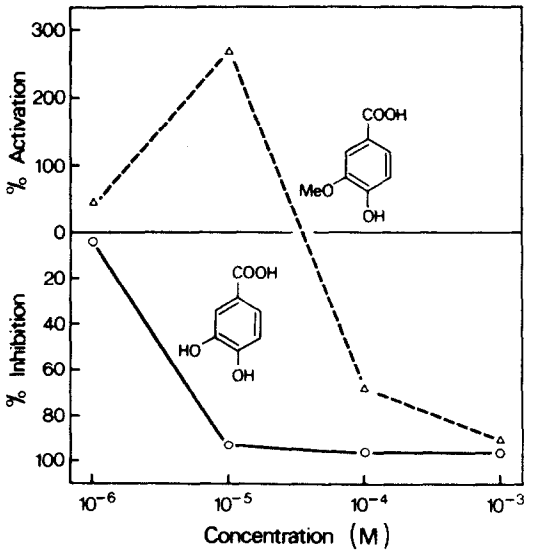

Fig. 1. Comparison of activities of 3,4-dihydroxybenzoic acid and 4 - hydroxy - 3 - methoxybenzoic acid in the horseradish peroxidase-catalysed decarboxylation of [1-^{14}C]IAA in the absence of DCP. The composition of the reaction mixture was the same as that of Table 1 and the concentration of the test compounds varied as indicated. $^{14}CO_2$ collected from the control had an average of 1430 dpm.

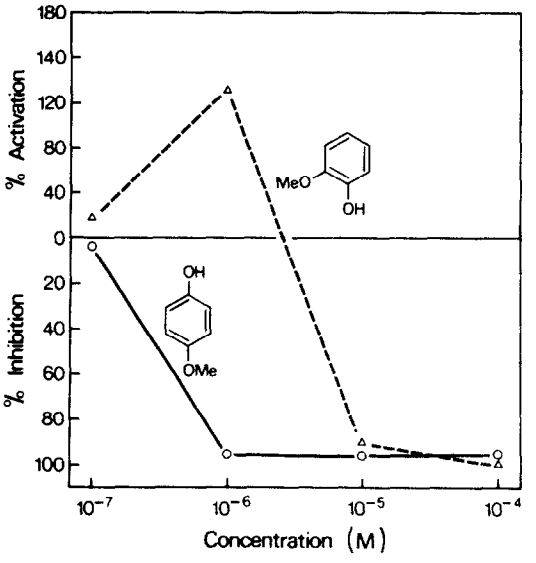

Fig. 2. Comparison of activities of guaiacol and *p*-methoxyphenol in the horseradish peroxidase-catalysed decarboxylation of [1-^{14}C]IAA in the absence of DCP. The composition of the reaction mixture was the same as that of Table 1 and the concentration of the test compounds varied as indicated. $^{14}CO_2$ collected from the control had an average of 1576 dpm.

some exceptions such as vanillin which was stimulatory over a broad concentration range (10^{-7}–10^{-3} M) and 4 - hydroxy - 3 - methoxyphenylacetic acid which was inhibitory at concentrations as low as 10^{-7} M (Table 1) [3].

p-*Diphenols and derivatives.* The three *p*-diphenols tested, like most of the *o*-diphenols, were strong inhibitors of IAA oxidation either in the presence or absence of DCP. *p*-Methoxyphenol (**35**) was as active as hydroquinone (**34**) and remained inhibitory at concentrations as low as 10^{-6} M, indicating that monomethylation had little or no effect on the activity. This contrasts to the effect of *O*-methylation on the activity of *o*-diphenols (Fig. 2).

m-*Diphenols and derivatives.* In the absence of DCP, most *m*-diphenols tested were cofactors of IAA oxidation with activity varying from slight to 28-fold activation (Table 1). Resorcinol (**39**) had higher activity than any of the substituted resorcinols. The cofactor activity of resorcinol in IAA oxidation has been reported with pineapple and wheat leaf extracts as the enzyme sources [7, 12]. Among the *m*-diphenols tested, 5 - acetyl - 2,4 - dihydroxyacetophenone (**47**) was an exception. It exhibited only a slight cofactor activity probably because both hydroxyls could form hydrogen bonds with adjacent carbonyl groups. *o*-Hydroxyacetophenone (**4**) and 2 - hydroxy - 6 - benzyloxyacetophenone (**51**) showed a similar activity. Compounds **44** and **50**, resulting from monomethylation of **43** and **49**, had almost no cofactor activity. In the presence of DCP, the *m*-diphenols produced slight to moderate inhibition of IAA oxidation probably by competition with DCP for the cofactor site. TLC indicated that the concentration of resorcinol was only partially decreased during IAA oxidation. Similar results were observed with 3,4-dihydroxyacetophenone. The sustained inhibition observed for resorcinol and 3,4-dihydroxyacetophenone in the presence of DCP can be attributed to the stability of these compounds. Resorcinol and 5-methylresorcinol have also been reported to show high stability in a lettuce phenolase system [13].

Substitution at position 2 on the aromatic ring significantly affected the activity. 2-Methylresorcinol (**48**) has a much weaker cofactor activity than resorcinol in the absence of DCP but a much higher inhibitor activity than resorcinol in the presence of DCP (Table 1). Such changes are further demonstrated by 2,6-dihydroxyacetophenone (**49**). This compound strongly inhibited IAA oxidation in the presence of DCP and either promoted or inhibited IAA oxidation in the absence of DCP, depending on the concentration used. A comparison of activities of 2,6-dihydroxyacetophenone and 2,4-dihydroxyacetophenone is shown in Figs. 3 and 4. This and other evidence (Table 1) indicate that substitution of resorcinol at the 2-position reduces the cofactor activity probably due to steric factors. The activity of 2,5-dihydroxyacetophenone is also shown in Figs. 3 and 4 to demonstrate further the difference in activity between *m*- and *p*-diphenols.

A comparison of the activity of 2,6-dihydroxyacetophenone and other 2,6-dihydroxy compounds (**52–55**) further indicates that steric factors are likely to be important. Increasing the size of the side-chain at the 2-position changed the activity from activation to

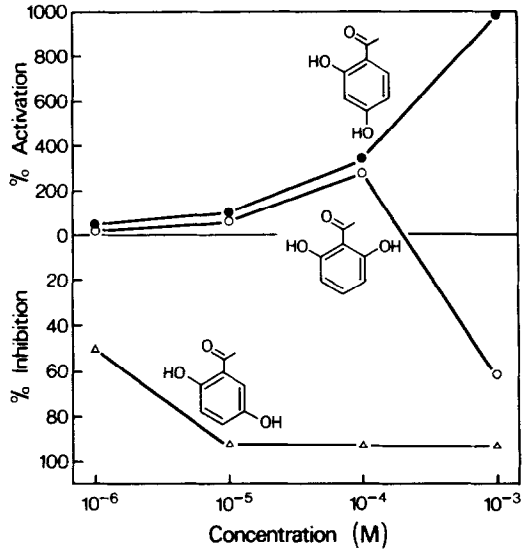

Fig. 3. Comparison of activities of 2,4-, 2,5- and 2,6-dihydroxyacetophenones in the horseradish peroxidase-catalysed decarboxylation of [1-^{14}C]IAA in the absence of DCP. The composition of the reaction mixture was the same as that of Table 1 except that the concentration of the test compounds varied as indicated. $^{14}CO_2$ collected from the control had an average of 1740 dpm.

inhibition (Table 1). The dual activity of 2,6-dihydroxyacetophenone (Fig. 3) suggests that this compound can bind to both cofactor and inhibitor sites; changes in this structure probably hinder binding to the cofactor site thereby accounting for the observed inhibition for compounds **53–55**. Introducing a bromine at the 3-position on the aromatic ring also drastically affected the activity. 3 - Bromo - 2,6 - dihydroxyacetophenone (**56**) strongly inhibited IAA oxidation either in the presence or absence of DCP (Table 1). Kinetic evidence showed that compound **56** was a temporary inhibitor whereas 2,6-dihydroxyacetophenone was a persistent inhibitor (Fig. 5). The inhibition kinetics have been confirmed by two other assays which measured the rate of decarboxylation of [1-^{14}C]IAA and the rate of dissappearance of free IAA.

Triphenols

Only five trihydroxyacetophenones or methyl ether derivatives thereof were tested (Table 1). 2,3,4- and 2,4,6-trihydroxyacetophenones were strong inhibitors of IAA oxidation either in the presence or absence of DCP. The activity of 2,3 - dihydroxy - 4 - methoxyacetophenone was similar to that of 2,3,4-trihydroxyacetophenone. However, the 4-methyl or the 4,6-dimethyl ethers (**60** and **61**) had activities quite different from that of 2,4,6-trihydroxyacetophenone.

In conclusion, the results further confirm that many phenols have a profound effect on the peroxidase-catalysed oxidation of IAA. The number of phenolic groups and their position relative to each other as well as to other substituents affect the activity. The following generalization appears to hold: (1) monophenols and *m*-diphenols produce activation when no

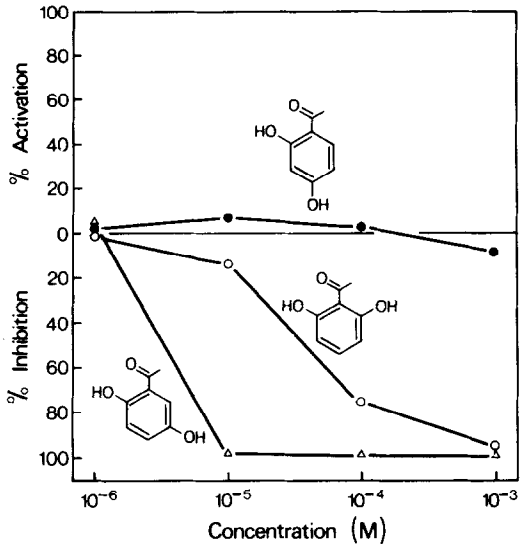

Fig. 4. Comparision of activities of 2,4-, 2,5- and 2,6-dihydroxyacetophenones in the horseradish peroxidase-catalysed decarboxylation of [1-^{14}C]IAA in the presence of DCP. The composition of the reaction mixture was the same as that of Table 1 except that the concentration of the test compounds varied as indicated. $^{14}CO_2$ collected from the control had an average of 42 654 dpm.

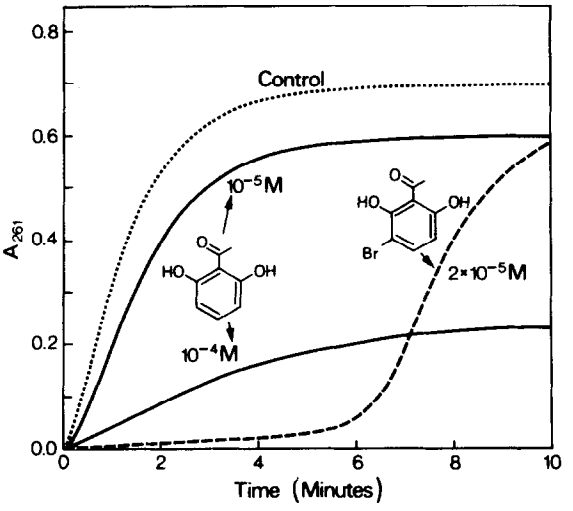

Fig. 5. Time course of IAA oxidation as influenced by 2,6-dihydroxyacetophenone and 3 - bromo - 2,6 - dihydroxyacetophenone. The composition of the reaction mixture was IAA (0.2 mM), DCP (0.05 mM), $MnCl_2$ (0.05 mM), K-Pi buffer (50 mM, pH 6.0), HRP (2 μg) and test compound (concentration as indicated) in a total of 3.0 ml. The reaction was run at 25° and the increase in A_{261} was recorded with an automatic recording spectrophotometer.

other cofactor is present. *p*-Substituted monophenols are more active than corresponding *o*- or *m*-derivatives; (2) *o*- and *p*-diphenols, with the exception of 3,4-dihydroxyacetophenone and 3,4-dihydroxypropiophenone, produce inhibition in the presence or absence of DCP. The inhibition by *o*-diphenols is temporary due to instability of these compounds in the IAA-oxidizing enzyme system; (3) in the absence of DCP, *o*-methoxyphenols and certain other phenols such as 3,4-dihydroxyacetophenone and 2,6-dihydroxyacetophenone either promote or inhibit IAA oxidation depending on concentration; (4) *m*-diphenols and 3,4-dihydroxyacetophenone produce a sustained inhibition when tested in the presence of DCP. From these observations it is evident that the prevailing concept of structure–activity relationships between phenolic compounds and IAA oxidation is an oversimplification.

Many phenolic compounds are natural constituents of plants. When tested *in vitro* they markedly affect the IAA-induced elongation of sections of oat internodes [14], oat coleoptiles [15], wheat coleoptiles [16] and pea internodes [15, 17]. Similar results were shown in the oat coleoptile curvature assay [18]. The importance of phenolic compounds in controlling growth has also been demonstrated in tobacco callus cultures by their action in promoting or inhibiting bud differentiation [19]. These effects were generally parallel to the cofactor or inhibitor activity of these compounds in the oxidation of IAA *in vitro* [4, 5, 17], and therefore the results obtained from growth assays were interpreted on the basis of regulation of IAA oxidation. The present study compares the activities of a larger number of phenols than used in any previous investigation. Therefore, it will be of interest to compare the relative activities of these compounds in IAA oxidation with their growth-regulating activities. The effect of natural phenolic compounds on IAA metabolism *in vivo* is difficult to determine because the composition, distribution and concentration of phenols in plant tissues are complex. More recently, however, some indication of a potential regulatory role of phenolic compounds in IAA metabolism has been given by their influence on formation of bound IAA and oxidative degradation of free IAA in maize stem sections [20].

EXPERIMENTAL

Assay for IAA oxidation. Oxidation of IAA was measured by three methods as previously described [21, 22] with minor modifications as indicated in Table 1: (1) decarboxylation of [1-^{14}C]IAA (60 μCi/μmol) determined by liquid scintillation counting of $^{14}CO_2$ collected from the reaction vessel by a Soluene®-saturated filter-paper wick during a 6-min period in a shaking water-bath at 25°; (2) the rate of product formation indicated by the increase in A at 261 nm; (3) the disappearance of IAA monitored by the modified Salkowski reagent. Stock solutions of the phenols tested were prepared in various concentrations with dimethyl sulfoxide and 5 μl were used for the assay. Dimethyl sulfoxide at this concentration had no effect on decarboxylation of [1-^{14}C]IAA. The composition and concentration of the reaction mixtures are described in Table 1 and Figs. 1–5. Because some phenols interfered with the last two assays, the activities of the phenols were compared mainly from the data obtained by the first method.

Chemicals. Compounds **23** and **53** were prepared by methylation (MeOH–H_2SO_4) of the respective benzoic acids and recrystallized before use. Compound **38** was prepared by treating 2,5-dihydroxyacetophenone in refluxing Me_2CO with MeI and dry K_2CO_3. The progress of the reaction was followed by TLC (Macherey–Nagel Si gel N-HR/UV_{254}) using $CHCl_3$–MeOH (19:1). Unreacted diphenol was separated by chromatography over silicic acid–celite (4:1) using CH_2Cl_2 as eluent. Recrystallization of the major fraction from petrol (35–60°) yielded 2 - hydroxy - 5 - methoxyacetophenone (**38**), mp 48–49° (lit. [23] 52°). Similarly, **50** was prepared from 2,6-dihydroxyacetophenone. Recrystallization of the product from MeOH–H_2O yielded 2 - hydroxy - 6 - methoxyacetophenone (**50**), mp 55–56° (lit. [24] 59–60°). Compound **51** was prepared in the same manner using benzyl bromide. Recrystallization of the crude product from Et_2O–petrol yielded 2 - hydroxy - 6 - benzyloxyacetophenone (**51**), mp 106–109° (lit. [24] 106–107°). Compound **56** was prepared from 2,6-dihydroxyacetophenone (170 mg) by stirring in THF (5 ml) with 1 equiv. 2-carboxyethyltriphenylphosphonium perbromide [25] for 17 hr at room temp. The product was chromatographed over a column of silicic acid–celite (4:1) using CH_2Cl_2 as eluent and recrystallized from MeOH–$CHCl_3$ yielding 3 - bromo - 2,6 - dihydroxyacetophenone (**56**), mp 140–142°; ^{1}H NMR (60 MHz, $CDCl_3$): δ 2.79 (3H, *s*, acetyl), 6.54, 7.56 (1H each, AB system, J = 9 Hz, H-4, H-5). Compounds **47** and **55** were prepared as described [26, 27]. Compounds **54**, **60** and **61** were synthesized by Dr. A. Stoessl of this centre.

Compounds **19** and **49** (Aldrich) and **24** (Pfaltz & Bauer) were purified by chromatography over a column of silicic acid–celite (4:1) using CH_2Cl_2 as eluent. Resorcinol (BDH) was recrystallized. Other compounds, used as received, were from the following sources: Aldrich (**4–7**, **9**, **13**, **15**, **17**, **22**, **27**, **29**, **33**, **35–37**, **40–46**, **52**, **57**, **59**); Eastman (**32**, DCP); Fisher (**1**, **12**, **34**); New England Nuclear ([1-^{14}C]IAA); Pfaltz & Bauer (**14**, **16**, **18**, **26**, **30**, **58**) and Sigma [**8**, **10**, **11**, **28**, **31**, **41**, IAA, HRP (type VI)].

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