Phytochemistry, Vol. 20, No. 9, pp. 2097–2100, 1981.
Printed in Great Britain.

0031-9422/81/092097-04 $02.00/0

EFFECT OF 3,4-DIHYDROXYACETOPHENONE AND SOME RELATED PHENOLS ON THE PEROXIDASE-CATALYSED OXIDATION OF INDOLE-3-ACETIC ACID

TSUNG T. LEE, ALVIN N. STARRATT and JOHN J. JEVNIKAR

London Research Centre, Agriculture Canada, University Sub Post Office, London, Ontario, N6A 5B7, Canada

(*Revised received* 20 *February* 1981)

Key Word Index—3,4-Dihydroxyacetophenone; acetovanillone; 3,4-dihydroxypropiophenone; *o*-diphenol; cinnamic acids; peroxidase; IAA-oxidase; indole-3-acetic acid.

Abstract—3,4-Dihydroxyacetophenone, 3,4-dihydroxypropiophenone and acetovanillone differed in activity in the enzymic oxidative reaction of indole-3-acetic acid (IAA) from *o*-diphenolic inhibitors, such as caffeic acid, 3,4-dihydroxyphenylacetic acid, protocatechuic acid and catechol. The first two ketones promoted IAA oxidation in the absence of the cofactor 2,4-dichlorophenol (DCP) whereas other *o*-diphenols inhibited it. 3,4-Dihydroxyacetophenone exhibits inhibitor activity only at high concentrations (10^{-3} M); methylation of the 3-hydroxyl did not change its activity, but monomethylation affected activity of other *o*-diphenols. In the presence of DCP, the two dihydroxyphenyl ketones caused persistent inhibition while the other *o*-diphenols induced only a delay in IAA oxidation.

INTRODUCTION

o-Diphenols such as caffeic acid, protocatechuic acid and catechol are strong inhibitors of enzymic oxidation of IAA. A common feature of the inhibition by these compounds is that they cause only a delay in IAA oxidation, after which the reaction resumes at the same rate as the control [1–4]. In this paper, however, we report that 3,4-dihydroxyacetophenone and 3,4-dihydroxypropiophenone have a different effect on the oxidation of IAA, indicating that the mode of inhibition of IAA oxidation by the caffeic acid-type compounds is not typical for all *o*-diphenols.

RESULTS AND DISCUSSION

Data presented in Table 1 compare the relative activity of ten *o*-diphenols as well as nine mono- or dimethyl derivatives. The regulatory activity in IAA oxidation was measured by decarboxylation of IAA catalysed by purified horseradish peroxidase in the presence or absence of the strong cofactor DCP. At 10^{-4} M concentration the activities of 3,4-dihydroxyacetophenone (**1**), acetovanillone (**2**) and 3,4-dihydroxypropiophenone (**3**) were different from those of others tested.

In the absence of DCP, compounds **1**–**3** were active as cofactors whereas compounds **5**–**18** were strong inhibitors. Methylation of the hydroxyl group at the 3-position of 3,4-dihydroxyacetophenone did not reduce the activity, but blocking both hydroxyl groups produced an inactive compound. The lower activity of 3,4-dihydroxypropiophenone compared to 3,4-dihydroxyacetophenone indicates that increasing the length of the side-chain decreases the activity, possibly because of a steric effect. However, **5**, resulting from replacement of one hydrogen of the side chain of 3,4-dihydroxyacetophenone with chlorine, produced inhibition instead of activation. This suggests that factors other than steric are also important since a chlorine atom is about the same size as a methyl group. Lanzarini *et al.* [5] reported that ascorbic acid was oxidized by an *o*-diphenol oxidase in the presence of **3** at a rate slightly greater than with catechol but that oxidation did not occur in the presence of 3,4-dihydroxy-ω-chloropropiophenone. However, the specificity of the *o*-diphenol oxidase and the enzyme used in this study towards other compounds was quite different.

In the presence of DCP, compounds **1**–**3** were less inhibitory than the others in the 6-min reaction period (Table 1) and showed persistent inhibition whereas the other *o*-diphenols did not. Previously, 2,6-dihydroxyacetophenone and a number of other phenols had been found to be persistent inhibitors [4, 6]. This difference in activities is demonstrated in Fig. 1 which compares the kinetics of inhibition induced by 3,4-dihydroxyacetophenone (**1**) and 3,4-dihydroxyphenylacetic acid (**14**) added before the start of the reaction. Compound **1** caused sustained inhibition while **14** caused only a lag. The results were confirmed by radioassay of $^{14}CO_2$ resulting from decarboxylation of [1-^{14}C]IAA and by colorimetric assay of IAA remaining after the reaction. When **14** was added 30 sec after the reaction had started, such a limited inhibition was not observed and the reaction was as rapid as without the inhibitor. Similar results had been noted previously with caffeic acid, ferulic acid, protocatechuic acid, scopoletin, 7-hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran, 3,7-dihydroxy-2,2-dimethyl-2,3-dihydrobenzofuran, and orchinol [3, 4, 6, 7]. In contrast, 3,4-dihydroxyacetophenone as well as 7-hydroxy-2,2-dimethyl-3-oxo-2,3-dihydrobenzofuran [6], 2,2-dimethyl-3-oxo-2,3-dihydrobenzofuranyl-7-*N*-methylcarbamate [6], 2,6-dihydroxyacetophenone [4] and 5-hydroxy-2,2-dimethylchromene [4] induced immediate and sustained inhibition even when added after the start of the reaction.

3,4-Dihydroxy-ω-chloroacetophenone (**5**), 3,4-dihydroxybenzonitrile (**8**), 4-methylcatechol (**9**), methyl 3,4-dihydroxybenzoate (**13**) and 3,4-dihydroxyphenylacetic acid (**14**) are new examples of *o*-diphenols which show inhibitor activity similar to caffeic acid. Ferulic acid, the monomethyl derivative of caffeic acid, is also a temporary inhibitor of IAA oxidase [2, 3, 8, 9]. A

Table 1. Comparison of activity of 3,4-dihydroxyacetophenone and related hydroxy- and methoxy-substituted benzenes on the enzymic decarboxylation of IAA in the presence or absence of 2,4-dichlorophenol (DCP)

No.	Compound	+DCP	−DCP	
		% Inhibition	% Promotion	% Inhibition
1	3,4-Dihydroxyacetophenone	81	380	
2	Acetovanillone	81	375	
3	3,4-Dihydroxypropiophenone	78	60	
4	3,4-Dimethoxyacetophenone	0	0	
5	3,4-Dihydroxy-ω-chloroacetophenone	100		99
6	Catechol	100		100
7	Guaiacol	100		100
8	3,4-Dihydroxybenzonitrile	100		99
9	4-Methylcatechol	100		100
10	Vanillyl alcohol	100		100
11	Protocatechuic acid	100		99
12	Vanillic acid	98		69
13	Methyl 3,4-dihydroxybenzoate	100		100
14	3,4-Dihydroxyphenylacetic acid	100		100
15	Homovanillic acid	100		99
16	Caffeic acid	100		100
17	Ferulic acid	100		100
18	3-Hydroxy-4-methoxycinnamic acid	99		68
19	3,4-Dimethoxycinnamic acid	0		0

The reaction mixture (2.0 ml) had 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) and peroxidase (2.5 μg). The compounds were also tested in the presence of 0.1 mM DCP. $^{14}CO_2$ collected from the control had an average of 1970 dpm without DCP and 64 550 dpm with DCP.

comparison of the inhibitor activities of caffeic acid and ferulic acid, **6** and **7**, **11** and **12**, **14** and **15** (Table 1) indicates that methylation of the hydroxyl group at the 3-position has little effect on the activity of these compounds at 10^{-4} M concentration. At lower concentrations, however, the *o*-methoxyphenols show activities varying from inhibition to activation (Table 2). Thus, compounds such as **7**, **12** and **17** can promote IAA oxidation at lower concentrations and inhibit IAA oxidation at higher concentrations. Methylation of both hydroxyl groups eliminates the activity.

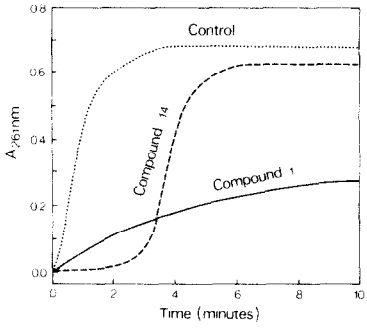


Fig. 1. Time course of IAA oxidation as influenced by 3,4-dihydroxyacetophenone (**1**) and 3,4-dihydroxyphenylacetic acid (**14**). The composition of the reaction mixture (3.0 ml) was 0.2 mM IAA, 50 μM each of 2,4-dichlorophenol and $MnCl_2$, 50 mM K-Pi buffer (pH 6), 0.1 mM of **1** or 8 μM of **14**, and 2 μg peroxidase. The inhibitors were added before the enzyme.

Sine 3,4-dihydroxyacetophenone is much weaker than DCP as a cofactor in IAA oxidation, the inhibition caused by it in the presence of DCP may be due to competition for the cofactor site. The extent of inhibition of IAA oxidation increased with increasing concentration of 3,4-dihydroxyacetophenone and reached a nearly complete inhibition at 10^{-3} M (Fig. 2). At this concentration the rate of IAA decarboxylation was 40% lower than that of the control without DCP. These results suggest that 3,4-dihydroxyacetophenone also binds to an inhibitor site when its concentration is above a certain level. This is supported by the data obtained in the absence of DCP showing that the cofactor activity of 3,4-dihydroxyacetophenone decreased sharply with increasing concentration above 10^{-5} M (Fig. 2). At a concentration of 10^{-3} M this compound was inhibitory. In this respect, the activity of

Table 2. Activity of low concentrations of *o*-methoxyphenols on the enzymic decarboxylation of IAA in the absence of DCP

Compound	% Promotion			% Inhibition		
	10^{-5}	10^{-6}	10^{-7} M	10^{-5}	10^{-6}	10^{-7} M
12	266	51	16			
7		125	15	90		
18		6	0	25		
10			14	90	0	
17			40	98	58	
15				80	33	8

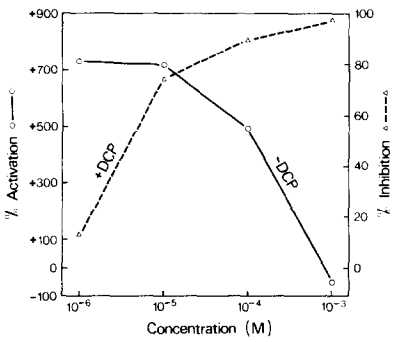


Fig. 2. Effect of concentration of 3,4-dihydroxyacetophenone on IAA oxidation in the presence or absence of the strong cofactor 2,4-dichlorophenol. The composition of the reaction mixture was the same as used in Table 1 except that varying concentrations of 3,4-dihydroxyacetophenone were used. The rate of IAA oxidation was determined by measuring decarboxylation of [1-^{14}C]IAA.

compound **1** is similar to certain *o*-methoxyphenols listed in Table 2.

Numerous phenolic compounds occur naturally in plants but their role in plant growth and metabolism remains uncertain. Many phenols showed significant effects on growth [10–12] and bud differentiation [11] *in vitro*. Such activities were attributed to influence in the IAA-oxidizing enzyme system; these and other activities of phenolic compounds were discussed in a recent review [13]. More recently, experiments with hydroxycinnamic acids and other phenols demonstrated that the phenols exerted a dual effect on IAA metabolism in maize stems [14]. They not only influenced the degradation of IAA but also the formation of bound IAA.

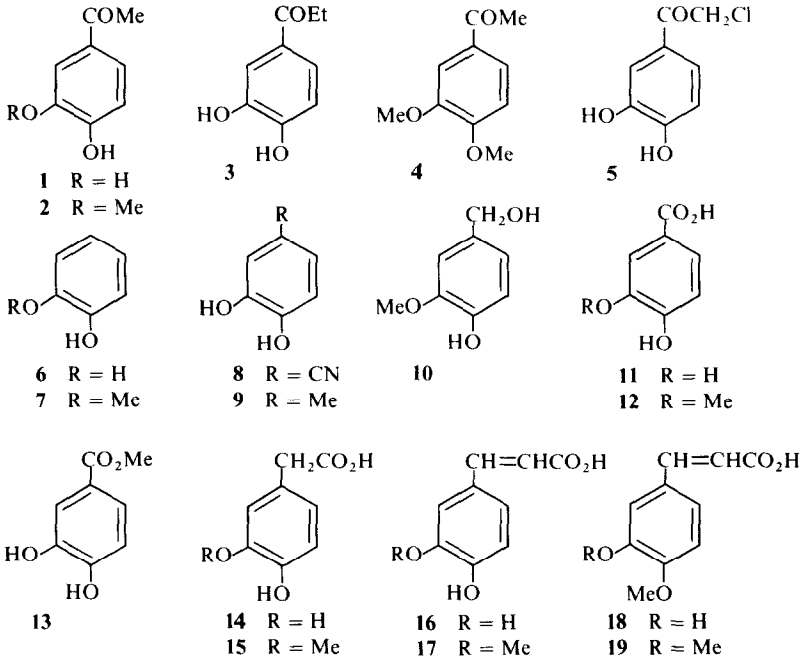
EXPERIMENTAL

Assay for IAA oxidation. Oxidation of IAA was determined by three methods as previously described [7, 15]. The first measured decarboxylation of [1-^{14}C]IAA (60 μCi/μmol, New England Nuclear) 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. The second recorded the rate of product formation as indicated by the increase in *A* at 261 nm with an automatic recording spectrophotometer. The third measured the disappearance of IAA by the modified Salkowski reagent. Some phenolic compounds interfered with the last two assays, therefore only phenols which did not cause interference were tested by the second and third methods. In all cases the temp. was 25°. The composition and concn of the reaction mixtures are given in the table and figures.

Chemicals. **1** was purchased from Pfaltz & Bauer and purified by chromatography over a column of silicic acid–celite (4:1) using CH_2Cl_2 as eluant followed by recrystallization from CH_2Cl_2–petrol (60–80°). **13**, prepared by methylation (MeOH–H_2SO_4) of 3,4-dihydroxybenzoic acid (BDH), was recrystallized from water before use. Other compounds, used as received, were from the following sources: **3**, **5** and **8** (Pfaltz & Bauer); **2**, **4**, **7**, **9**, **10**, **12**, **15**, **18** and **19** (Aldrich); **6** (Fisher); **11** (BDH); **14**, **16**, IAA and horseradish peroxidase (EC 1.11.1.7, type VI) (Sigma); **17** and DCP (Eastman).

REFERENCES

1. Waygood, E. R., Oaks, A. and Maclachlan, G. A. (1956) *Can. J. Botany* **34**, 905.
2. Sacher, J. A. (1963) *Am. J. Botany* **50**, 116.
3. Lee, T. T. (1977) *Plant Physiol.* **59**, 372.
4. Lee, T. T., Starratt, A. N., Jevnikar, J. J. and Stoessl, A. (1980) *Phytochemistry* **19**, 2277.
5. Lanzarini, G., Pifferi, P. G. and Zamorani, A. (1972) *Phytochemistry* **11**, 89.
6. Lee, T. T. and Chapman, R. A. (1977) *Phytochemistry* **16**, 35.
7. Lee, T. T., Rock, G. L. and Stoessl, A. (1978) *Phytochemistry* **17**, 1721.
8. Gelinas, D. A. (1973) *Plant Physiol.* **51**, 967.
9. Mudd, J. B. and Burris, R. H. (1959) *J. Biol. Chem.* **234**, 3281.



10. Nitsch, J. P. and Nitsch, C. (1962) *Ann. Physiol. Vég.* **4**, 211.
11. Lee, T. T. and Skoog, F. (1965) *Physiol. Plant.* **18**, 386.
12. Tomaszewski, M. and Thimann, K. V. (1966) *Plant Physiol.* **41**, 1443.
13. Harborne, J. B. (1980) in *Secondary Plant Products* (Bell, E. A. and Charlwood, B. V., eds.) p. 329. Springer, New York.
14. Lee, T. T. (1980) *Physiol. Plant.* **50**, 107.
15. Lee, T. T. (1977) *Can. J. Botany* **55**, 574.