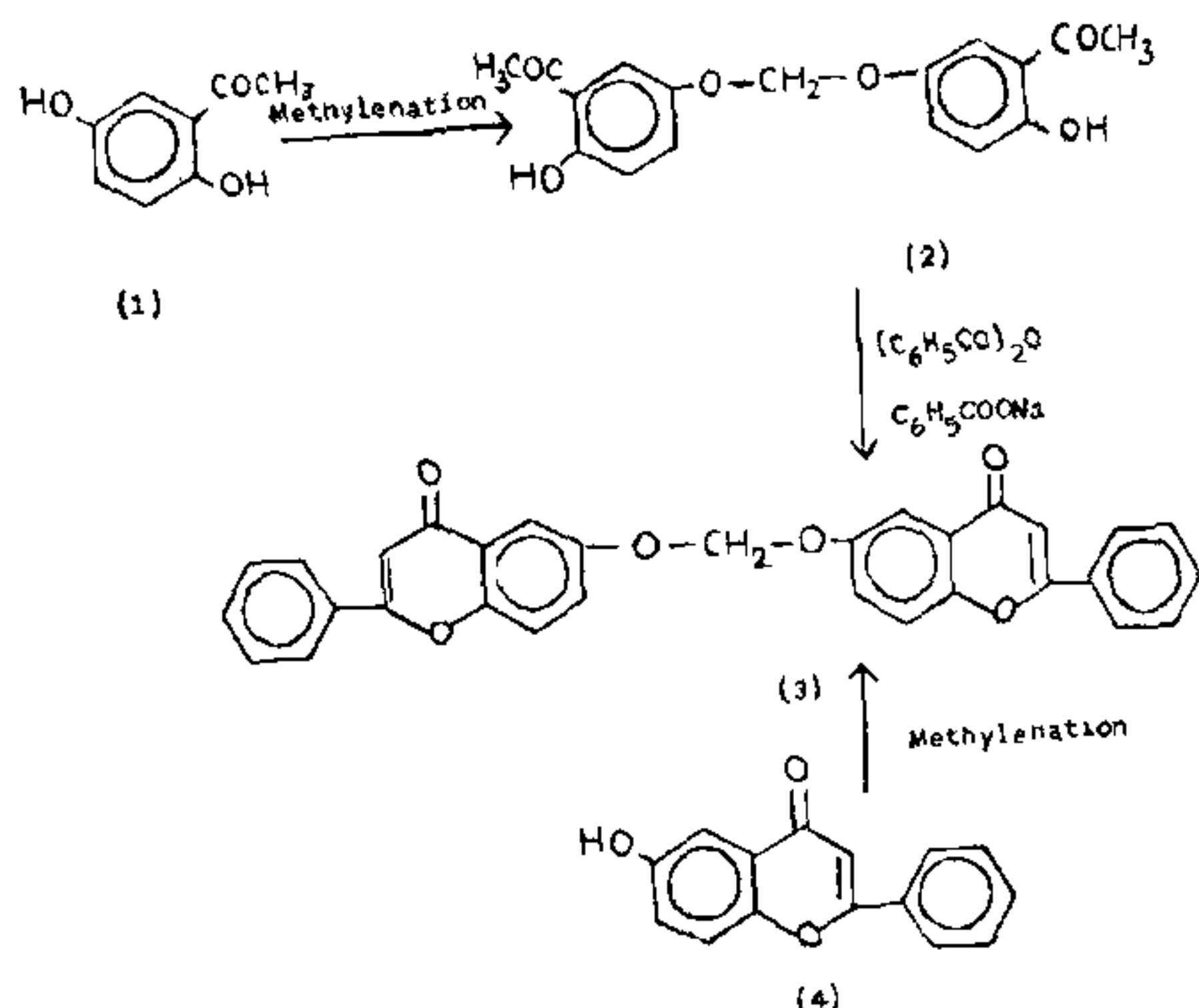


flavone (4), by mixed melting point, co-chromatography and comparison of their infrared spectra.



EXPERIMENTAL

Bi-(2-hydroxy-1-acetophenonyl-5-oxy) methane.—Quinacetophenone (2 g) was refluxed in acetone (40 ml) with methylene iodide (1 ml) and anhydrous potassium carbonate (10 g) for 4 hrs. Acetone was distilled off and water (100 ml) added to the residue. The solid, after filtration and drying, crystallized from ethanol as colourless needles, m.p. 192–193° ν_{\max} 1630 cm^{-1} (H-bonded C=O). It gave positive methylenedioxy test (found C, 64.45; H, 5.0, $\text{C}_{17}\text{H}_{16}\text{O}_6$ required C, 64.6; H, 5.1%).

Synthesis of bi-(6-flavonyloxy) methane:

(1) **from bi-(2-hydroxy-1-acetophenonyl-5-oxy) methane.**—A mixture of bi-(2-hydroxy-1-acetophenonyl-5-oxy) methane (2.6 g) benzoic anhydride (35 g) and sodium benzoate (7 g) was heated at 180–185° for 4 hrs. It was then cooled and the pale brown mass was broken up and added to alcohol (170 ml). The mixture was boiled during the gradual addition of potassium hydroxide (18 g) dissolved in 40 ml of water and then heated for 30 minutes under reflux. The alcohol was removed by distillation. The residue was dissolved in water. The filtered solution was saturated with carbon dioxide. The precipitate was filtered, dried and then crystallized from ethyl acetate as colourless microprisms, m.p. 236–237°. $R_f = 0.65$ chloroform-ethyl acetate (3 : 20) ν_{\max} 1640 cm^{-1} (flavone C=O) (found C, 75.95; H, 4.0; mol. wt. (Rast method), 469; $\text{C}_{31}\text{H}_{20}\text{O}_6$ requires C, 76.21; H, 4.1%; mol. wt., 488).

(2) **Dimer from 6-hydroxyflavone.**—6-hydroxyflavone (0.2 g), acetone (125 ml), methylene iodide (0.1 ml) and anhydrous potassium carbonate (4 g) were refluxed together for 40 hrs. Acetone was

distilled off and water (100 ml) was added to the residue. The solid (0.1 g), after filtration and drying, crystallized from ethyl acetate as colourless microprisms, m.p. 236–237°. Mixed m.p. with the compound synthesised by the method (1) was not depressed. It gave positive methylenedioxy test. $R_f = 0.65$; chloroform-ethyl acetate (3 : 20) [found C, 76.1; H, 4.1; mol. wt. (Rast method), 471; $\text{C}_{31}\text{H}_{20}\text{O}_6$ requires C, 76.21; H, 4.1%; mol. wt., 488].

Methylenedioxy test.—The methylenedioxy test was performed by heating the compound with gallic acid and conc. H_2SO_4 on a boiling water bath. Emerald green colour was taken as a positive reaction.

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EFFECT OF ELECTROLYTES ON THE RATE OF HYDROLYSIS OF 1-PHENYLNEOPENTYL CHLORIDE IN WATER

THE diagnostic introduced by Ingold¹ of adding a lyate ion or another nucleophile for distinguishing between $\text{S}_{\text{N}}1$ and $\text{S}_{\text{N}}2$ mechanisms has been criticised by Hine². Following this, this technique has either been ignored³ or misinterpreted⁴. However, Schleyer has recently proved the correctness of Ingold's view, so far as azide ions are concerned⁵. We have now studied a system, which is not readily susceptible to $\text{S}_{\text{N}}2$ attack, using the less nucleophilic bromide as the reagent.

1-phenylneopentyl chloride has been shown to solvolyse by the $\text{S}_{\text{N}}1$ mechanism, even in relatively non ionizing solvents^{6,7}. It has also been shown that its structure disfavors $\text{S}_{\text{N}}2$ attack⁸. We have

studied the kinetics of hydrolysis in solvent water in which, because of the high ionizing capacity of the medium, an S_N2 pathway is even less likely. Our results (Table I) show that non-common ion

TABLE I

Effect of added ions on the rate of hydrolysis of 1-phenylneopentyl chloride in water

Temp. 45–70°C (RCI) = 0.0006 M

Added salt	Concentration (M)	$10^4 k_1 (s^{-1})$	% increase in rate due to added salt
Nil	..	1.47	..
LiClO ₄	0.05	1.47	0.0
NaNO ₃	0.05	1.52	2.7
NaBr	0.01	1.63	10.9
"	0.05	2.04	38.8
"	0.10	2.15	46.3

electrolytes show negligible ionic strength effect, which is consistent with the Debye-Hückel theory. Addition of bromide ions on the other hand shows marked increase in rate; the non-linearity between the increase in rate and the bromide ion concentration clearly indicates that its effect is not due to the normal type of salt effect described by Winstein⁸. Further support for this conclusion comes from the observation that the rate constants in the presence of bromide ions increase with the progress of reaction. This effect is not seen either in the absence of bromide ions or in the presence of other non-common ion electrolytes.

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THE OBSERVED ACTIVATION OF ENZYMES BY Mn^{++} IS SOMETIMES AN ARTIFACT

DURING a study of the effect of metal ions on the activity of a bacterial malic dehydrogenase (EC 1.1.1.37), it was found that Mn^{++} at concentrations as low as 10^{-5} M stimulated the rate at which absorption at 340 nm increased when malate and NAD were in excess. When oxaloacetate (OAA) and NADH were in excess, addition of Mn^{++} reduced the rate at which absorption at 340 nm decreased (Fig. 1). The kinetics of these reactions suggested the presence of a contaminating activity such as malate-NAD-oxido-reductase (EC 1.1.1.38). This enzyme, which is activated by Mn^{++} , catalyzes the conversion of malate to pyruvate and carbon dioxide¹.

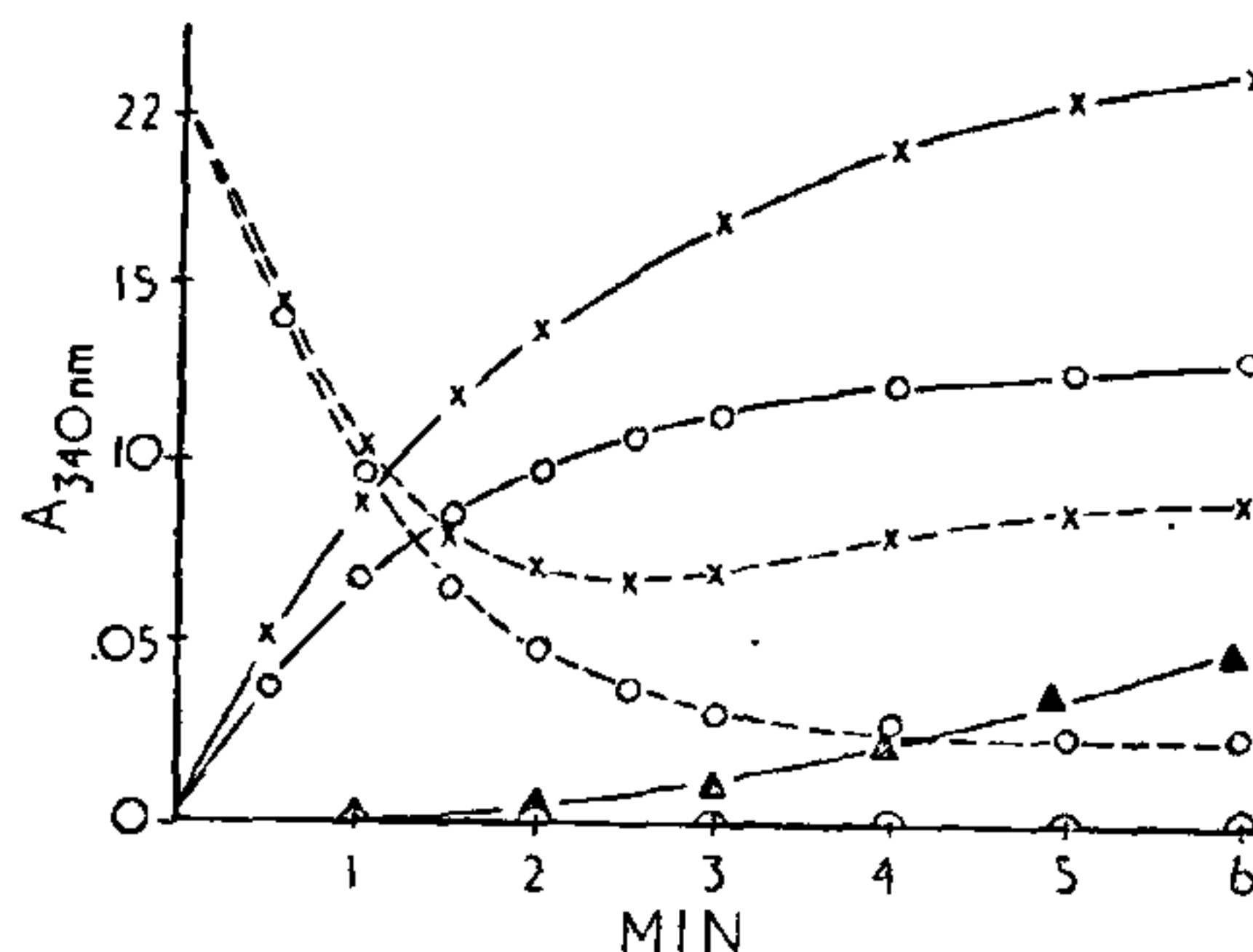


FIG. 1. Effect of Mn^{++} on a malic dehydrogenase (EC 1.1.1.37). Assays were done spectrophotometrically at 340 nm. Dotted lines: enzyme with oxaloacetate and NADH in tris buffer at pH 8.5. Solid line with circular symbols: enzyme with malate and NAD at pH 8.5. Curves with X symbols contained in addition 1 mM Mn^{++} . Curve with triangles: buffer only plus 1 mM Mn^{++} at pH 8.5. Base line (half circles): buffer at pH 7.0 plus 1 mM Mn^{++} , or buffer at pH 8.5 with 1 mM EDTA plus 1 mM Mn^{++} , or buffer alone.

We sought direct evidence for the presence of this enzyme by testing for the formation of both pyruvate and CO_2 , the latter was determined manometrically after acidification. The CO_2 resulted from the spontaneous decomposition of OAA and its amount was independent of the Mn^{++} concentration. What pyruvate was detected by gas-liquid chromatography (GLC) was also unrelated to metal ions and was traced, in part, to the breakdown of OAA during methylation for GLC. Pyruvate was also estimated with lactic dehydrogenase; no significant increase could be attributed to the presence of Mn^{++} in the malic dehydrogenase assay. Further protein purification