when unsaturated fats are added to the diet (1,2), but Wilson and Siperstein(3,4) found no consistent difference in fecal excretion of C^{14} by rats fed 20% corn oil or 20% lard and injected intravenously with cholesterol-4-C^{14}. Both groups, however, excreted a larger amount of injected C^{14} than did rats fed a fat-free diet. The amount of C^{14} in non-digitonin-precipitable sterols was greatly increased in animals on the corn oil diet as compared to rats receiving either no fat or a 20% lard diet. In rats fed these fats at 30% of the diet the latter observation was confirmed and in addition, it was noted that in 4 out of 5 groups total C^{14} recovery in feces was greater in rats receiving corn oil. In our experiments using 20% fats in the diet the rats excreted about 52% of administered C^{14} in 14 days. This compares favorably with data of Wilson and Siperstein(3,4) in the experiments using fat at 20% of the diet. Fractionation of the C^{14} excreted in the feces was not included in our experiment. It is apparent that in these rats the effect of kind of fat fed is on type of sterol excreted rather than on total amount.

In studies of the influence of dietary fat on serum cholesterol levels it is necessary to ascertain the possible simultaneous effect of the diet on tissue deposition of cholesterol. Alfin-Slater *et al.*(9) found an accumulation of cholesterol in livers and adrenals of rats on an essential fatty acid-deficient diet with a corresponding decrease in serum cholesterol levels. Addition of essential fatty acids to the diet caused a reversal of this situation. The data presented in Table II indicate that under the conditions of our experiments varying the type of fat in the diet for a 4- to 5-week period did not influence amount of C^{14} deposited in livers or pooled organs (heart, lungs, aorta, and kidneys) after intravenous injection of cholesterol-4-C^{14}.

Summary. The influence of a purified diet containing 20% fat as hydrogenated cottonseed oil, corn oil, or coconut oil on fecal excretion and tissue deposition of C^{14} has been studied in rats injected with cholesterol-4-C^{14}. There was no significant difference between the 3 groups in amount of C^{14} excreted in feces or deposited in liver and in pooled internal organs (heart, lungs, aorta, and kidneys).

1. Hellman, L., Rosenfeld, R. S., Insull, W., Jr., Ahrens, E. H., Jr., *J. Clin. Invest.*, 1957, v36, 898.

2. Gordon, N., Lewis, B., Eales, L., Brock, J. F., *Lancet*, 1957, v2, 1299.

3. Wilson, J. D., Siperstein, M. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 113.

4. ———, *Am. J. Physiol.*, 1959, v196, 596.

5. Meier, J. R., Siperstein, M. D., Chaikoff, I. L., *J. Biol. Chem.*, 1952, v198, 105.

6. Siperstein, M. D., Chaikoff, I. L., *ibid.* 1952, v198, 93.

7. Entenman, C., Lerner, S. R., Chaikoff, I. L., Dauben, W. G., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 364.

8. Sperry, W. M., Webb, M., *J. Biol. Chem.*, 1950, v187, 97.

9. Alfin-Slater, R. B., Aftergood, L., Wells, A. F., Deuel, H. J., Jr., *Arch. Biochem. and Biophys.*, 1954, v52, 180.

Received July 20, 1959. P.S.E.B.M., 1959, v102.

Polarographic and Biologic Activities of N-Substituted Maleimides.* (25176)

ROBERT M. MUIR†

Dept. of Botany, University of Iowa, Iowa City

Friedman, Marrian and Simeon-Reuss(1) discovered that the maleimide molecule has antimitotic properties and forms –SH adducts readily. Recently(2) N-ethylmaleimide has been found to react with a number of thiols and thiol esters and the reaction

* Investigation supported by grant G-45 from Nat. Science Fn.

† The author gratefully acknowledges advice and guidance of Dr. Stanley Wawzonek in measuring half-wave potentials and the technical assistance of Dr. Leslie Paleg.

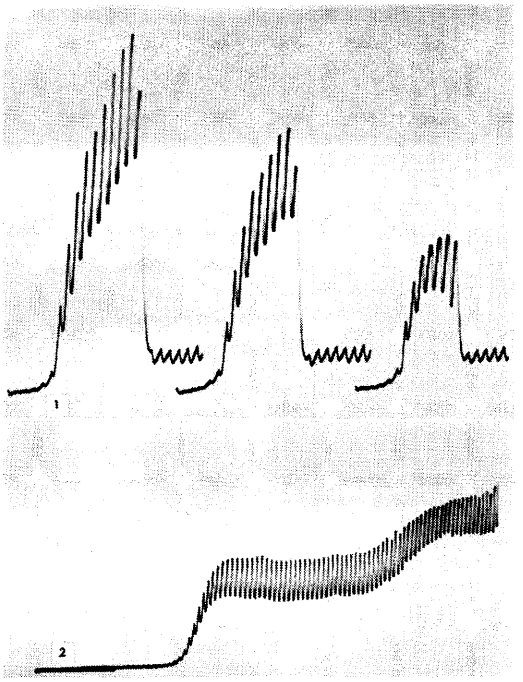

FIG. 1. Polarogram of oxygen maximum in the electrolyte solution without maleimide (left) and oxygen maxima following additions of one and 2 aliquots (5 μl) of 5×10^{-3} M N-phenylmaleimide.

FIG. 2. Polarogram of reduction of N-(2,4-dichlorophenyl)maleimide. Ordinate: current. Abscissa: voltage.

has been used in chromatography of such compounds. In examining defoliation and growth effects of sulfhydryl reagents van Overbeek, Blondeau and Horne(3) employed substituted maleimides and reported that chlorination of the ring of N-phenylmaleimide increased its effect in inhibiting growth, and saturation of the double bond of the maleimide ring abolished such effects. The increase in activity due to chlorination of the ring was attributed to increase in lipoid solubility favoring penetration through cell membranes. It is also possible that reactivity of the double bond is affected by substitution in the phenyl ring and inhibition of growth is related to ease with which addition of –SH to the unsaturated system takes place. Wawzonek and Fossum (4) found the ease of reduction of unsaturated carbonyl system of esters and amides of β-aroylacrylic acid as measured by the dropping mercury electrode, parallels the antibacterial activity of the compounds. Reduction of maleimides at the dropping mercury electrode shows that substituents in the phenyl ring do affect reactivity of the maleimide double bond.

Materials and methods. Polarograms were obtained with a Sargent Model XII Recording Polarograph. With increasing applied e.m.f. in presence of oxygen an increased reduction current flow is obtained, reaching a maximum (oxygen maximum) in these determinations at –0.4 volt and then dropping sharply to the diffusion current level of the electrolyte solution (Fig. 1). The effects of maleimides‡ upon oxygen maximum were measured in 10 ml of 0.01 N KCl with 8% methyl alcohol. Aliquots of maleimide dissolved in electrolyte-methyl alcohol solution were added to the electrolysis vessel until the maximum was depressed to less than one-half of its value in the original electrolyte solution. The drop time was 7.2 seconds with bath temperature of 24°C. Shunt ratio was 50 and span electromotive force was 4 volts. Amount of maleimide required to depress oxygen maximum by exactly one-half is the half-suppression value (H.S.V.) for the compound in micromoles/liter. Reduction of an organic molecule at the dropping mercury electrode gives rise to a "polarographic wave" in the current-voltage measurement (Fig. 2). Standard practice reports polarographic reduction potential as the potential of the midpoint of the wave where the current is equal to half its limiting value. This potential is termed the half-wave potential ($E_{1/2}$) and is referred to the saturated calomel electrode at 25°C. Half-wave potentials for maleimides were measured in a solution of 0.1M tetrabutylammonium iodide and 0.001% methyl red in 50% *p*-dioxane by volume. Dioxane was freshly purified. Maleimide concentration was 4 millimolar, drop time was 4.3 seconds, shunt ratio was 50 and span electromotive force was 2 volts. Electrolysis vessels were gassed with nitrogen for 30 minutes before obtaining the current-voltage curves. Biologic activity of maleimides in repressing elongation of cells was determined by a standardized procedure in which growth of 3 mm segments of Avena coleoptile tissue induced

‡ Purified samples kindly supplied by Dr. J. van Overbeek of Shell Development Co.

TABLE I. Half-Suppression Values, Half-Wave Potentials and Growth Inhibition for N-Substituted Maleimides.

Compound	H.S.V.,* μmole/l	$-E_{1/2}$ (S.C.E.),† v	% inhibition of IAA-induced growth by maleimide‡ IAA 2×10^{-7}M	IAA 2×10^{-5}M
N-Isopropylmaleimide	>110	.89	1	1
N-Phenylmaleimide	3.9	.80	25	15
N-(4-Chlorophenyl) maleimide	1.8	.76	35	20
N-(2,4-Dichlorophenyl) maleimide	.55	.76	65	50
N-(2,4,6-Trichlorophenyl) maleimide	.55	.74	100	100

* Amount of maleimide required to reduce maximum current flow due to oxygen by one-half.
† Half-wave potential with reference to saturated calomel electrode.
‡ Maleimide concentration 5×10^{-5}M in all cases.

by indole-3-acetic acid (IAA) in 0.8% isopropyl alcohol solutions was measured after 12 hours.

Results. The effects of substances upon the oxygen maximum may be used as a relative measure of their adsorbability or surface activity(5), a small H.S.V. thus indicates high adsorbability or surface activity. Within the series of maleimides in Table I there is an extreme variation in H.S.V., the value for the N-isopropyl compound being very large while di- and trichloro- compounds have values only a little larger than the lowest value yet obtained (0.3 for 4-chloroindole-3-acetic acid). Water solubility of these compounds(3) varies in the same direction as surface activity but the magnitude of variation in solubility is at least 5 times greater (N-isopropyl->5,000 ppm, N-phenyl- 75-100 ppm, N-(4-chlorophenyl)- and N-(2,4-dichlorophenyl)- 5 ppm.

No reduction waves for N-phenylsuccinimide at potentials less than –2.0 v S.C.E. were found and the waves recorded for maleimides therefore represent reduction of the carbon-carbon double bond. Heights of waves (I_d = 0.85 and 0.5 for N-(2,4-dichlorophenyl) maleimide, Fig. 2) point to a 1-electron change for each wave. The half-wave potential of the first wave, measures the relative ease of electron additions to the double bond and reflects electrical and steric effects present in the molecule. Addition of RS^- would be subject to similar effects. The half-wave potential of the second wave would have no significance for the biologic addition reaction but was –1.49, –1.3, –1.25, –1.3, and –1.32 v respectively, for compounds listed in Table I. The values of half-wave potentials for the first wave show that the chemical group on the N-atom of the maleimide structure affects reduction of the double bond with the aromatic ring increasing ease of reduction considerably and the 2,4,6-trichloro-substitution resulting in maximum effect.

Growth effects of maleimides at 5×10^{-5}M concentration are recorded in Table I. At this concentration N-(2,4,6-trichlorophenyl) maleimide prevents completely the growth that may be induced by the growth hormone, indole-3-acetic acid (IAA). N-isopropylmaleimide at this concentration has a minimum perceptible effect represented by 1% inhibition. With higher concentrations of the maleimides, inhibition is complete even for N-isopropylmaleimide (4×10^{-4}M).

Discussion. Explanations of differences in biologic activity of a group of analogs such as the series of maleimides examined usually emphasize solubility or lipophilic characteristics rather than chemical reactivity. Since little is known of the functional significance of the former and the latter is usually entirely unknown the arbitrary choice cannot be criticized. Even where information is more complete the basis for activity as related to structure may be obscure. For the 5 maleimide compounds the differences in biologic activity correspond generally to differences in water solubility, lipophilic properties, adsorbability or surface activity, and ease of reduction at the maleimide carbon-carbon double bond. However, this correspondence simply relates the several properties as codependent on molecular constitution.

The most effective compound, N-(2,4,6-trichlorophenyl)maleimide, is virtually insol-

uble in water although very dilute solutions may be prepared by first dissolving the compound in a small quantity of isopropyl alcohol. Since N-isopropylmaleimide is more than 5,000 times as soluble in water, yet causes 100% inhibition of growth at only an 8-fold increase in concentration, it is unlikely that oil/water solubility properties are responsible for the differences in inhibition. Similarly the considerable difference in the H.S.V. of the isopropyl compound compared to those of the most effective compounds indicates little dependency of inhibition effects on adsorbability or surface activity.

The chemical reactivity of maleimides appears the logical basis for their biologic activity. The molecular configuration which can add –SH more readily at the carbon-carbon double bond is more effective as inhibitor of the growth response. Thus, N–(2,4,6-trichlorophenyl)maleimide with the most positive half-wave potential is the most effective inhibitor. A half-wave potential more negative by 20 millivolts results in less inhibitory effect and the least inhibitory effect is given when the half-wave potential is more negative by 150 millivolts. The fact that the same half-wave potentials were obtained for mono- and di-chloro-compounds may be attributed to limitations of the method in measuring potential differences as small as 10 millivolts. It is also possible that with the same half-wave potential the greater inhibition by the dichloro-compound as compared with N–(4-chlorophenyl)maleimide results from its greater adsorbability or surface activity. Yet the more important effect of chemical reactivity is demonstrated by the dichloro- and trichloro-compounds having the same H.S.V. but different half-wave potentials and different degrees of inhibition.

Some indications of a specificity of maleimides for sulfhydryls involved in the growth process were obtained by van Overbeek, *et al.* (3) when they found that 5×10^{-5} M N–(2,4-dichlorophenyl) maleimide inhibited growth by 50% while reducing respiration only 20% and N-isopropylmaleimide inhibited growth 23% and respiration only 5%. Such specificity, if real, would have its foundation in critical chemical reactivity as indicated by measurements of the half-wave potentials.

Summary. The half-suppression values and half-wave potentials for 5 N-substituted maleimides were measured with the polarograph and compared with their inhibitory effects on growth of plant tissue induced by indoleacetic acid. The chemical group at the N-atom of the maleimide structure determines the ease of reduction of the carbon-carbon double bond. This chemical reactivity appears to be the basis for the biologic activity of maleimides with some possibility of adsorbability or surface activity playing a minor role.

1. Friedmann, E., Marrian, D. H., Simeon-Reuss, I., *Brit. J. Pharmacol.,* 1949, v4, 105.

2. Benesch, R., Benesch, R. E., Gutcho, M., Laufer, L., *Science,* 1956, v123, 981.

3. van Overbeek, J., Blondeau, R., Horne, V., *Am. J. Bot.,* 1955, v42, 205.

4. Wawzonek, S., Fossum, J. H., *Sbornik, mezinarod. polarog. sjlzdu praze,* 1st Cong., 1951, Pt. I. Proc., 548.

5. Kolthoff, I. M., Lingane, J. J., *Polarography,* Interscience Publishers, N. Y., 1952.

Received March 24, 1959. P.S.E.B.M., 1959, v102.