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REACTION OF MALFORMIN WITH SULFHYDRYL COMPOUNDS*

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Abstract—Malformin reacts with the sulfhydryl compounds, cysteine and β-mercaptoethanol, to form an insoluble 1:1 addition product. This reaction may explain the ability of thiol compounds to inhibit the activity of malformin and suggests reaction of malformin with thiol compounds *in vivo*.

INTRODUCTION

A VARIETY of sulfhydryl compounds, such as cysteine, glutathione, and 2,3-dimercaptopropanol, inhibit the promotion of corn root curvatures by malformin.[1] We recently observed precipitation in solutions containing both malformin and cysteine, and report here on the nature of this reaction.

RESULTS AND DISCUSSION

When malformin A, a mixture of 83 per cent A_1 (I) and 17 per cent A_2 (II), was mixed with an excess of cysteine or β-mercaptoethanol in an aqueous solution containing dimethylsulfoxide, an amorphous substance began to precipitate within 10 min. Precipitation appeared complete after 24 hr. The substance obtained from malformin and cysteine or β-mercaptoethanol was insoluble in 1 N NaOH or 1 N HCl, and in many organic solvents, but dissolved in trifluoroacetic acid. Dilution of solutions in trifluoroacetic acid with water resulted in immediate precipitation. The malformin–cysteine compound reacted with ninhydrin, indicating the presence of an amino group, but the malformin–β-mercaptoethanol substance did not. Neither compound reacted in the nitroprusside test for sulfhydryl groups; both reacted in the test for the disulphide group.[2] Both compounds remained at the origin when chromatographed on paper using phenol: water (7:3) or *n*-butanol–acetic acid–water (4:1:2) and on TLC plates using ethyl acetate saturated with water. The compounds were detected with ninhydrin, iodine or nitroprusside for disulphide. Neither malformin A_1 nor A_2 was detected by TLC of the addition products, indicating the latter was not contaminated with the former.

The molecular weight of the malformin–cysteine substance determined by the Signer method, was approximately 700. Elemental analysis was in agreement with that of a mixture of $C_{26}H_{46}O_7N_6S_3$ (malformin A_1–cysteine, m.w. 651, 83 per cent) and $C_{25}H_{44}O_7N_6S_3$ (malformin A_2–cysteine, m.w. 637, 17 per cent) for the malformin–cysteine substance, and

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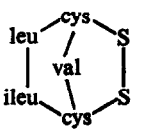
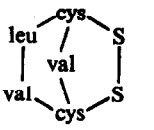
[2] G. TOENNIES and J. J. KOLB, *Anal. Chem.* **23**, 823 (1951).



(I) (A_1)



(II) (A_2)

with that of a mixture of $C_{25}H_{45}O_6N_5S_3$ (malformin A_1–β-mercaptoethanol, m.w. 608, 83 per cent) and $C_{24}H_{43}O_6N_5S_3$ (malformin A_2–β-mercaptoethanol, m.w. 594, 17 per cent) for the malformin–β-mercaptoethanol substance. These formulae represent 1:1 addition products of malformin A_1 and A_2 with the thiol. When the malformin–cysteine adduct was oxidized with performic acid, cysteic acid was detected by paper chromatography; performic acid oxidation of malformin did not liberate cysteic acid.

Thiol-disulphide exchange reactions are known to occur when disulphides, *XS–SX*, are mixed with thiols, *RSH*, as in reactions (1) and (2).[3] When the cyclic disulphide malformin, $\overline{MS\text{–}SM}$, is

$$XS\text{–}SX + RSH \rightleftharpoons XS\text{–}SR + XSH \quad (1)$$

$$XS\text{–}SR + RSH \rightleftharpoons RS\text{–}SR + XSH \quad (2)$$

mixed with a thiol, *RSH*, reaction (3) appears likely. If the

$$\overline{MS\text{–}SM} + RSH \rightarrow \overline{MSH \quad MS\text{–}SR}\downarrow \quad (3)$$

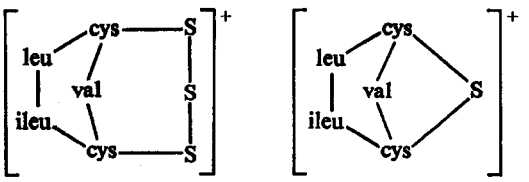
1:1 addition product of malformin and thiol is insoluble it will precipitate readily. We suggest that the reaction of malformin with thiols is expressed in reaction (4) and that the addition product has the structure III.

(I) (A_1) (III) (4)

R = $CH_2CH(NH_2)COOH$ or CH_2CH_2OH

The mass spectra of malformin–cysteine and malformin–β-mercaptoethanol were essentially identical. Major ion peaks in the higher mass region were *m/e* 561, 547, 529, 515, 497 and 483. *m/e* 529 (A_1^+) and *m/e* 515 (A_2^+) are indicative of ions of malformin A_1 (I) and A_2 (II), respectively. *m/e* 561 indicates the presence of malformin A_1 plus one sulphur ($A_1^+ + 32$), *m/e* 547 is A_2 plus one sulphur ($A_2^+ + 32$), *m/e* 497 is A_1 minus one sulphur ($A_1^+ - 32$), and *m/e* 483 is A_2 minus one sulphur ($A_2^+ - 32$). The ions *m/e* 561 and 497, which are not found in the mass spectrum of malformin itself, may be illustrated as IV and V or their equivalents, respectively. The ions, "three-sulphur malformin" (IV), "two-sulphur malformin" (I), and "one-sulphur malformin" (V) presumably originate from compound III. The parent peaks, *m/e* 650 (malformin A_1–cysteine) and *m/e* 636 (malformin A_2–cysteine) or *m/e* 607 (malformin A_1–β-mercaptoethanol) and *m/e* 593 (malformin A_2–β-mercaptoethanol), may not have been observed because of ion instability. Insolubility of the addition products may also have precluded the nitroprusside test for sulfhydryl groups.

[3] N. KHARASCH, *Organic Sulfur Compounds*, Vol. 1, p. 87, Pergamon Press, New York (1961).



(IV) (V)

Both addition products were tested for biological activity in the corn root curvature test.[4] Because of their insolubility, they were used as a suspension in trifluoroacetic acid and water. Their biological activity was approximately 1/10 that of malformin itself.

When 2 μmoles of malformin (1 mg in 1 ml) were mixed with 0·2 or 1 μmole of cysteine in 0·1 and 0·5 ml of water, and allowed to stand for 24 hr, approximately 75 and 93 per cent, respectively, of the original biological activity in the supernatant was lost. Similar results were obtained using β-mercaptoethanol. If malformin reacts only by a 1:1 addition with the thiol, the decline in activity should have been no greater than 10 and 50 per cent, respectively. Under conditions of limiting thiol quantities, the reaction of malformin with thiols may be more complex than simple 1:1 addition.

To determine if thiols inhibit malformin *in vivo*, corn seeds were germinated in the presence of various concentrations of β-mercaptoethanol for 32 hr, rinsed, transferred to Petri dishes containing filter paper moistened with 0·1 mg/l. malformin, and incubated for 40 hr. Pre-treatment with 8×10^{-3} M β-mercaptoethanol reduced approximately 80 per cent corn root curvatures induced by malformin. In similar experiments, cysteine pre-treatment had no effect.

A close relationship between sulfhydryl compounds and plant-growth processes has been demonstrated.[5] Numerous growth regulators or inhibitors combine with sulfhydryl groups. α,β-Unsaturated lactones, which inhibit plant growth,[6,7] react with cysteine and related compounds with the formation of ring compounds.[8] Maleic acid, which combines with numerous thiols,[9] reacts with protein thiol groups and thereby inhibits succinic dehydrogenases.[10] 2,3,5-Triiodobenzoic acid, which inhibits auxin transport, reacts with glutathione, presumably forming a thio-ether. Maleimids, iodoacetate and *p*-chloromercuribenzoic acid react similarly.[11] Heliangine, a natural growth inhibitor which promotes root formation, forms an addition product with cysteine.[12] The reaction of malformin with thiols by thiol-disulphide exchange differs from the reaction of other growth regulators with sulfhydryl compounds. The malformin reaction explains, in part, the ability of sulfhydryl compounds to inhibit malformin activity. Furthermore, the reaction offers insight for studies concerning the mode of action of malformin.

EXPERIMENTAL

Malformin–Cysteine Addition Product

L-Cysteine, 121 mg (1 mmole) in 5 ml water, was added to malformin A, 53 mg (0·1 mmole), in 50 ml dimethylsulfoxide–water (1:9, v/v). Precipitation was observed within 10 min. After 24 hr the amorphous

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precipitate was centrifuged, washed with water and 0·5 N HCl to remove cysteine, filtered through sintered glass, washed, and dried over NaOH. $[\alpha]_D^{25} = +135°$ (after 1 hr), $+141°$ (after 5 days), $+131°$ (after 10 days) ($c. = 1$, CF_3CO_2H). Because the optical rotation did not change with time, the possibility that malformin had been converted to less-active conformation III, as reported earlier,[13] was excluded. The mol. wt. (approx 700) was determined by the Signer method using CF_3CO_2H as solvent and azobenzene as standard. Found: C, 47·77; H, 6·93; N, 12·66; S, 14·70. Required for a mixture of $C_{26}H_{46}O_7N_6S_3$ (83%) and $C_{25}H_{44}O_7N_6S_3$ (17%), the 1:1 addition products, respectively, of malformin A_1 and A_2 with cysteine: C, 47·84; H, 7·09; N, 12·96; S, 14·83. Relative intensity of mass spectrum peaks were 3·1 (*m/e* 594), 21 (*m/e* 561), 4·8 (*m/e* 547), 100 (*m/e* 529), 20 (*m/e* 515), 57 (*m/e* 497) and 14 (*m/e* 483). *m/e* 594 remained unexplained, presumably due to impurities. Malformin also reacted with cysteine when methylcellosolve–water or acetic acid–water were used as solvents.

Malformin–β-mercaptoethanol Addition Product

β-Mercaptoethanol, 78 mg (1 mmole) in 5 ml water, was added to maformin A, 53 mg (0·1 mmole), in 50 ml dimethylsulfoxide–water (1:9 v/v). After 24 hr the precipitate, 38 mg, was processed as described. $[\alpha]_D^{25} = +167°$ (after 1 hr), $+170°$ (after 5 days), $+186°$ (after 15 days) ($c. = 0·66$, CF_3CO_2H). Found: C, 49·55; H, 7·46; N, 11·71; S, 15·70. Required for a mixture of $C_{25}H_{45}O_6N_5S_3$ (83%) and $C_{24}H_{43}O_6N_5S_3$ (17%), the 1:1 addition products, respectively, of malformin A_1 and A_2 with β-mercaptoethanol: C, 49·25; H, 7·43; N, 11·57; S, 15·88. Relative intensity of mass spectrum peaks were 19 (*m/e* 561), 3·3 (*m/e* 547), 100 (*m/e* 529),17·5 (*m/e* 515), 80·5 (*m/e* 497), and 17 (*m/e* 483).

Biological Activity of the Addition Products

Malformin–cysteine addition product, 20 mg, was dissolved in 2 ml CF_3CO_2H. 0·2 ml of the solution was added slowly with stirring to 20 ml water, forming a suspension of the adduct (100 ppm). The suspension was diluted and used in various concentrations in the corn root curvature assay.[4] The optimum concentration of the adduct, which induced curvatures on more than 80% of the roots, was 0·8 and 1·6 ppm; the optimum concentration of malformin A is approximately 0·1 ppm. The solvent, CF_3CO_2H, had no effect at concentrations of the adduct below 10 ppm, but was inhibitory at higher concentrations. The malformin–β-mercaptoethanol addition product was tested in the same manner with similar results.

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