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their hypothetical character these considerations were not included in the short communication.

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## BIOLOGY

## Conversion in Plants of Benzoic Acid to Salicylic Acid and its Bd-Glucoside

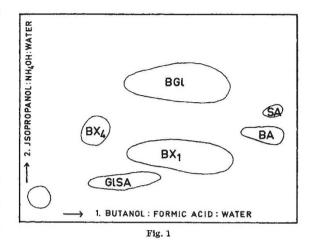
PREVIOUS investigations of the auxin metabolism in plant tissues led to the following findings of a ring hydroxylation resulting in 2-hydroxyindolyl-3-acetic acid1,2, and a hydroxynaphthalene-1-acetic acid, which probably hydroxylated in the 8-position, and their glucosides<sup>2</sup>. Other known products of the auxin metabolism were the aspartic acid conjugates<sup>8</sup> and the  $\beta d$ -glucose esters<sup>4,5</sup> of the free auxinic acids. Analogues of the last two metabolites have also been described for benzoic acid<sup>4,6</sup>. In wheat coleoptiles no hydroxylation of benzoic acid could be demonstrated.

Using the method described earlier<sup>4</sup> etiolated Helianthus hypocotyls were incubated with  $6 \times 10^{-5}$ and  $6 \times 10^{-4}$  molar carboxyl-labelled benzoic acid. Hypocotyls were crushed directly on chromatographic paper 'S and S 2043b'. The solvents used in chromatography were butanol / formic acid / water (10:1:2) for the first and isopropanol / ammonium hydroxide / water (80 : 15 : 5) for the second direction. The compounds labelled with carbon-14 were then detected by autoradiography of these paper chromatograms (Fig. 1). Besides the very large spot of the benzoyl-\$d-glucose (BGl) and two hitherto unknown compounds  $(BX_1 \text{ and } BX_4)$ , it shows the free benzoic acid (BA), a very small spot of salicylic acid (SA), and a relative large spot of  $\beta d$ -glucosido-salicylic acid (GlSA). The glucoside was characterized by hydrolysis with emulsin. The hydrolysis products agreed with co-chromatographed authentic salicylic acid and glucose. The radioactivity and colour reaction with ferric chloride, and diazobenzene-sulphonic acid were in accordance with this phenolcarbonic acid.

Recently' gentisic and o-pyrocatechuic acid were found in leaves of various plants fed with salicylic acid. The same acids and p-hydroxybenzoic acid were formed in plants incubated with carboxyl-labelled benzoic acid<sup>7</sup>. Whether they did occur as free acids or as glycosides is questionable, because the investigations were carried out with acid-hydrolysed ethanol extracts of plant tissues. Helianthus annuus hypocotyls, Solanum tuberosum tubers, and Pisum sativum internodes and roots are able to form salicylic acid, and in much larger quantities  $\beta d$ -glucosidosalicylic acid from benzoic acid. No other phenolcarbonic acids or their glycosides in traceable amounts derived from benzoic acid.

In wheat coleoptiles no ortho hydroxylation of benzoic acid could be demonstrated. On the other hand, it was shown that wheat plants are capable of metabolizing salicylic acid to gentisic and o-pyro-catechuic acids?. Therefore, it seems that ortho hydroxylation of aromatic acids needs a specific reaction mechanism, while further hydroxylation appears a rather widespread and more unspecific reaction, presumably catalysed by polyphenoloxidases.

In the biosynthesis of coumarin<sup>8</sup> a high rate of conversion of cinnamic acid to Bd-glucosido-o-



coumaric acid was found. This glucoside has been discussed as a possible intermediate in the biosynthosis of coumarin. The same situation has been described for the synthesis of hydroxynaphthalene-1acetic acid<sup>2</sup> and here of salicylic acid. But whether the glucosido-phenolcarbonic acids are intermediates or detoxification products is unknown. Due to its lactone ring, coumarin may be an exception; however, other phenolcarbonic acids are substrates for phenoloxidases. In the case of salicylic acid gentisic and pyrocatechuic acid, and possibly polyphenols, are formed. The glucosides of phenolcarbonic acids prevent this oxidation. Therefore, it seems that this property may be the general function of those phenolglucosides. A balance favourable for this assumption is indicated by the fact that the quantitative relation between the free phenolcarbonic acids and their glucosides (about 1 : > 10) was always nearly the same, whether plants were fed with the free phenolcarbonic acids or their glucosides. The function of these glucosides is under investigation.

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- <sup>1</sup> Klämbt, H. D., Naturwiss., 46, 649 (1959).
- <sup>2</sup> Klämbt, H. D., Planta. 57, 391 (1961).
- <sup>6</sup> Andreae, W. A., and Good, N. E., Plant Physiol., 30, 380 (1955).
  <sup>6</sup> Klämbt, H. D., Planta, 56, 618 (1961); 57, 339 (1961).
- Zenk, M. H., Nature, 191, 493 (1961).
- <sup>4</sup> Andreas, W. A., and Good, N. E., *Plant Physiol.*, 32, 566 (1957).
  <sup>7</sup> Ibrahim, R. K., and Towers, G. H. N., *Nature*, 184, 1803 (1959).
- <sup>8</sup> Brown, S. A., Towers, G. H. N., and Wright, D., Canad. J. Biochem. Physiol., 38, 143 (1960).

## Antagonistic Effect of Urea and 2-Chloroethanol on the Resistance to High Salt Concentration in Wheat Seedlings

IT is considered that the ammonia produced by the hydrolysis of urea in the soil damages the roots of plants. Recently, Cooke1 reported that at least one other factor may be operative in urea toxicity. Weber and Tanford<sup>2</sup> demonstrated that 2-chloro-ethanol strengthened the intramolecular hydrogenbonding of ribonuclease and urea possessed the opposite character. The increase of resistance to high salt concentration by the seed treatment with dilute 2-chloroethanol solution was found by Miyamoto<sup>3</sup>.