Botany. — *On the Chemical Nature of the Rootforming Hormone.* By KENNETH V. THIMANN and F. W. WENT. (From the William G. Kerckhoff Laboratories of the Biological Sciences California Institute of Technology, Pasadena, California). (Communicated by Prof. F. A. F. C. WENT).

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The assay method described by WENT in the preceding paper affords the possibility of investigating the chemical nature of the rootforming hormone, rhizocaline. For such experiments the first essential is to find a good source of the substance. In the experiments of BOUILLENNE and WENT (1933) it was found that an extract of rice polishings had considerable activity. When starting experiments on the chemical nature of rhizocaline, however, using the above method of assay, a number of other preparations were tested and some richer sources than the rice polishing extract were found. The rootforming hormone appears to be fairly widely distributed in nature; some of our determinations on various products are listed in Table I, in which the activity in rhizocaline and in auxin may

TABLE I.

Root producing and growth promoting activities of various products. Activities expressed per mg of fresh weight or solution.

Product	Activity RU/mg	Activity GS/mg	Activity Ratio RU/GS
Rice polishings, water extract	1	0.25, 0.5	2—4
Urine at different times of day	0.7—3.0	0.5	2- 6
Wheat germ oil, extracted with $KHCO_3$	0.036	0.035	1
"Galen B"	3.3	2.7	1
Pollen of Acer Negundo	0	20	0
„ „ Hicoria cordiformis	2.5	65	0.04
„ „ Phalaris minor	1	0.4	2.5
„ „ Sequoia sempervirens (1934)	2.5	0.5	5
„ „ Juniperus	9	0.5	18
„ „ Quercus alba.	60	0.7	85
Leaves of Helianthus annuus.	0.02	0	∞
„ „ Prunus Laurocerasus.	0.04	0	∞
Leaves of Malva	0.1	0.1	1
Etiolated Pisum buds and shoots	0	0.01	—

be compared. In general those preparations active in inducing rootformation are also active in promoting cell elongation. The search was further shortened by the discovery that in particular the partially purified extract of growth substance (auxin) obtained from *Rhizopus suinus* culture medium, and another extract prepared by Prof. Kögl from urine were highly active. From the method by which these preparations were obtained it was clear that the rootforming hormone must be, like auxin (auxenolic acid) an organic acid. Experiments were therefore carried out to determine whether the properties of the two hormones were similar or not.

The most characteristic property of the auxins is the ease with which they are inactivated by oxidising agents, the double bond of the molecule being very susceptible to attack. A series of oxidations was carried out with the urine preparation mentioned above, and referred to in this and the preceding paper as KV. Table II shows that rhizocaline is inactivated

TABLE II.

Inactivation of Rhizocaline by small amounts of oxidising agents.

Treatment		Activity in original solution RU. cc $\times 10^3$	Activity after oxidation RU. cc $\times 10^3$
H_2O_2	2×10^{-4} mols per mg	15.5	2.2
H_2O_2	9×10^{-4} mols per mg	8	8
I_2	1×10^{-4} mols per mg	8	0.0
$KMnO_4$	3×10^{-6} mols per mg	8	0.0
Bz_2O_2	ca. 10^{-4} mols per mg	8	7

by H_2O_2, $KMnO_4$ in neutral solution, and iodine, but not by benzoyl peroxide (also not by H_2O_2 in one experiment) under the conditions used here. Rhizocaline is therefore probably an unsaturated acid. Treatment with hydroxylamine gave no loss of activity.

Experiments to determine the dissociation constant of the acid by extracting with a given solvent at different pH (cf. DOLK and THIMANN, 1932) have so far given only approximate results. However, in Table III are given results of a series of extractions from buffered solutions, each extraction being made 3 times with ½ the volume of $CHCl_3$. It may be seen that the pH range involved is between 3 and 6. Direct determinations indicated a partition coefficient between $CHCl_3$ and water of about 2, and from this figure and the assumption of a pK of 4.5 the percentages of the hormone which should be extracted were calculated. It may be seen that the agreement is fairly good, and hence a partition coefficient of about 2 and a log dissociation constant pK = 4.5 are approximately correct. These properties are similar to those of the auxin from RHIZOPUS (P = 1.6; pK = 4.75).

TABLE III.

Extraction of Rhizocaline at different pH. Three extractions with half volume of $CHCl_3$ extract residue brought to the same final volume. Calculations for P = 2, pK = 4.5.

Activities found in RU cc	pH 6.0	5.0	4.0	3.0	2.2
Extract	0.8	5	8	7	7
Residue.	6.0	9	1.4	0.5	0.0
Activity of extract in % . . .	11.8	36	85	93	100
Activity of extract Calculated %	9.3	30	60	87	100

One of the last stages in the purification of the auxins is vacuum distillation, in which, with the still here used, the bulk of the growth promoting activity is found in the fraction distilling at 90—100° and 0.001 mm Hg. The distribution of rhizocaline in the fractions of one distillation was found to be parallel with the distribution of growth substance, the fraction at 90—100° containing the principal amounts of both.

Finally the formation of lactone by heating with 1 % HCl in MeOH as described by KÖGL, HAAGEN—SMIT and ERXLEBEN (1933) was determined for both hormones. Under the conditions used, conversion of the acid to a neutral compound was 98 % complete for rhizocaline and 97% complete for auxenolic acid.

Extraction of the partially purified syrups with various organic solvents also showed a qualitative parallelism in the distribution of the two hormones between extract and residue.

It follows that rhizocaline is an unsaturated organic acid, of about the same acid strength and solubilities as the *Rhizopus* auxin and auxin A, and distilling and lactonising (or esterifying) under the same conditions. The two hormones are thus closely similar, but not necessarily identical, particularly since the results of Table I show very wide discrepancies in the ratio of root units to growth substance units in a given preparation.

In order to further investigate the relationship we were able, through the generosity of Professor KÖGL, to test the activity of his three crystalline auxins in rootformation. Auxin A had only a low activity, when received, for rhizocaline, and was almost inactive for growth substance. The activities of auxin B and heteroauxin, on the other hand, were high; the data are listed in Table IV, together with the activities of the stock solutions of KV and the *Rhizopus* preparation Br. F. It may be seen that though the activities are roughly parallel, the value of the ratio: rhizocaline units per mg auxin units per mg is not constant. This points to the possibility that the two hormones are not identical but are both present in all these preparations.

Further evidence on this point was obtained from a series of oxidations with hydrogen peroxide. Treatment of a very dilute solution with 8 mols of H_2O_2 inactivated auxin B, as determined on *Avena*, by about 90 %, and heteroauxin less than 50 %; in rootformation the inactivation was 90 % and 70 %. Treatment with 40 mols of H_2O_2 produced complete inactivation of both substances for both functions.

TABLE IV.

Comparison of auxin and rhizocaline activity of KOGL's crystalline auxin-products

Activities in 10^4 units mg.

	Rhizocaline	Mean	Auxin	Rhizocaline auxin
Auxin A	0.5	0.5	< 0.01	> 50
Auxin B	10, 7, 17	11	23	0.48
Heteroauxin	2.6, 2.0, 4.0 (or 21)	2.9 (8.5)	28	0.1 (0.3)
KV (containing auxin A)		4.9 ± 1.1	0.9	5.5
Br. F.		3.6 ± 0.85	1.2	3.0

The inactivation of the rootforming activity was thus different in auxin B from that in heteroauxin, which would seem to indicate that the rootforming activity is not due to one substance present as impurity in both preparations. However, the other alternative, that the root-producing and growth-promoting hormones are identical, is not supported by the wide variations in the ratio of the two activities. In this respect it seems especially significant that in the purification of the auxin the activity in rootformation is reduced by from 10 to 50 times. It is at least safe to conclude that the 2 hormones are extremely closely related. It is hoped to obtain conclusive evidence as to their identity or difference at a later date.

LITERATURE CITED.

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