The Cucumber Tendril – a New Test Organ for Gibberellic Acid

There is an evergrowing interest in gibberellic acid (GA) and other gibberellins, both for their possible practical application in agriculture[1], and as an aid in the solution of theoretical problems of growth and flowering in higher plants.

As a part of an investigation of the genetics and physiology of the cucumber[2], started at the Plant Genetics Section of the Weizmann Institute of Science, it was intended to study the role of GA. Thus, a simple method for the estimation of gibberellic acid-like substances in plant extracts was looked for.

The possibility of using cucumber tendrils as test organs for GA, became apparent in an experiment in which the tendrils of young, GA-treated, plants appeared several days earlier than those of control plants. It was first found that 10 mm sections of straight tendrils, when incubated for about 24 h in solutions of 1·0 ppm. GA, 50 ppm. GA, 1·0 ppm. indoleacetic acid (IAA), 50 ppm. IAA, and in control solution, had a final length of 14·7 ± 0·3, 15·0 ± 0·3, 14·0 ± 0·2, 14·5 ± 0·2 and 13·9 ± 0·3 mm respectively. Here it is of particular interest that GA and IAA immersed tendril sections differed more in their form than in their length. While those incubated in GA were still straight or somewhat bent, those incubated in IAA were twisted (see Fig. 1). Similar twisting was observed with indolebutyric acid and indolepropionic acid.

The effect of incubation period was further tested. Fifteen 10 mm sections were incubated for 6, 21 and 44 h in small glass vials containing control, 1 ppm. and 10 ppm. GA solutions. Results presented in table form show only slight elongation after 6 h, but significant increase in length over the control as effected by the 1·0 and 10·0 ppm. GA solutions, after 21 h. Although still further elongation can be detected after 44 h, the difference between the 1·0 and 10·0 ppm. solutions as observed in this and other tests seem to decrease, when the incubation period is much more than 24 h.

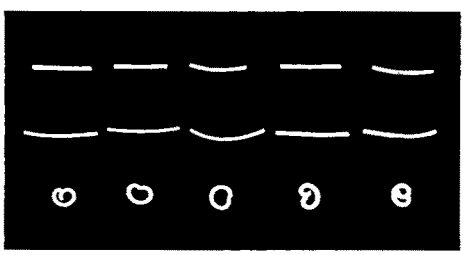

Fig. 1.—Three groups of five 10 mm cucumber tendril sections, incubated in different solutions: upper group—control; middle group—1·0 ppm. GA solution; and lower group—1·0 ppm. IAA solution.

In another test the reaction of different regions along the tendril to various concentrations of GA was investigated. Sections from tendril regions 25–35, 35–45, 45–55, and 55–60 mm from the tip were incubated in control, 0·33 ppm., 1·0 ppm., 3·3 ppm. and 10·0 ppm. GA solutions for 24 h. Each tendril region and each concentration was

Elongation of 10·0 mm sections of cucumber tendrils incubated for 6, 21 and 44 h in control, 1·0 ppm. and 10·0 ppm. GA solutions. Numbers indicate mm final length.

GA concentrations	Incubation time in h		
	6	21	44
Control	11·7 ± 0·14	12·4 ± 0·20	12·9 ± 0·15
1·0 ppm. GA. .	11·7 ± 0·08	13·3 ± 0·23	14·7 ± 0·10
10·0 ppm. GA .	12·0 ± 0·14	14·0 ± 0·33	14·9 ± 0·48

tested by 15 (tendril region 25–35 mm from the tip) or 20 (all other regions) sections. Results and LSD values for each tendril region are presented in Figure 2.

It can be seen that as the distance from the tip increases the elongation decreases; moreover, the elongation ability of the '55–60 mm' tendril region is too small to serve as tester for GA. The optimum of elongation, for the three other regions, is near to 3·3 ppm. and a continuous increase of elongation from 0·33 ppm. to 3·3 ppm. GA as well as a decrease from 3·3 to 10·0 ppm. GA is apparent.

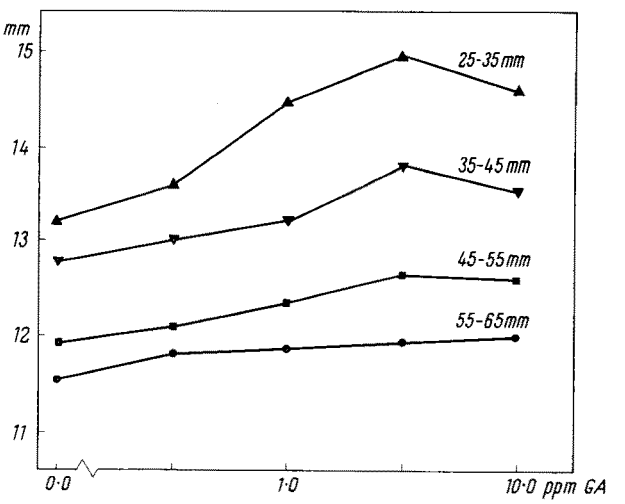

Fig. 2.—The effect of GA concentration on 10·0 mm sections taken from various regions along the tendril. Results are expressed in mm final length. The LSD values for the 25—35 mm, 35—45 mm, 45—55 mm, and 55—60 mm regions were 0·51 mm, 0·43 mm, 0·28 mm, and 0·35 mm respectively.

Based both on the data presented above and on those of other similar tests, the following method was adopted. Tendrils were harvested from greenhouse-grown cucumber plants of inbred lines (*Beit Alpha*, selfed for seven generations). A daily crop of about 25 straight tendrils, 13–14 cm long, was received from every 100 plants. Tendrils can be harvested from the fourth to the eighth week after emergence. From every tendril 10 mm sections of two regions (25–35 and 35–45 mm from the tip) were cut. Five sections of the same region were put in every 55 × 15 mm glass vial. The glass vials contained 1·0 ml of a buffer-sucrose solution (1·019 g citric mono hydrate, 1·749 g Potassium phosphate and 30·0 g sucrose/l) and a 50 × 15 mm piece of filter paper to which a certain amount of GA was applied by means of a micropipette, or which was cut out after paper chromatographic separation. Every amount of GA was tested with five sections of each of the two regions and the test was repeated at least once.

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Comparing this test to others recently suggested, some advantages of the present method seem to be apparent. PHINNEY *et al.*[3] suggested maize dwarf mutants as test plants; the tested material is to be applied by means of drops on intact leaves and results 'read' after several days. With this method, as well as with the cucumber leaf and the dwarf pea seedlings methods[4], the paper strips, representing different *Rf* values, after chromatographic separation, cannot be tested directly but must be eluted and reconcentrated before application. The wheat leaf sections test of RADLEY[5] can be used directly with filter paper strips but only qualitative values are obtained. Since the cucumber tendril test has a simple procedure, gives fair quantitative estimates of GA and can be combined directly with paper chromatographic separation of plant extracts, its further use is being advocated.

The author is indebted to Miss R. SMILANSKI-DROR for her skillful technical help. The GA sample was donated by Dr. P. W. BRIAN of the Imperial Chemical Industries, England.

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Résumé

On peut se servir de tronçons de vrilles de concombre en voie de dévelopment *in vitro*, pour déterminer d'après leurs réactions la présence d'acide gibberellique (GA). L'avantage de cette méthode réside dans le fait qu'on peut l'employer en la combinant avec la chromatographie sur papier et ainsi évaluer le GA dans l'extrait des plantes.

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Vobasin und Voacryptin, zwei neue Alkaloide aus *Voacanga africana* Stapf

Kürzlich isolierte RAO[1] aus der Apocynacee *Voacanga africana* Stapf die Alkaloide Voacafrin und Voacafricin, die mit einer spektralen Absorption bei 240 und 315 mμ gegenüber den bis jetzt bekannten Voacanga-Alkaloiden Voacangin[2,3], Voacamin[3,4], Vobtusin[4], Voacorin[5], Voacamidin[6], Voacristin[6] und Voacangarin[7] einen neuen Spektraltyp repräsentieren. Diese Arbeit veranlasst uns zu der Mitteilung, dass wir aus *Voacanga africana* Stapf ebenfalls ein neues Alkaloid dieses Spektraltyps isoliert haben, für das wir den Namen Vobasin vorschlagen. Zu seiner Isolierung chromatographierten wir eine aus Stammrinde in üblicher Weise gewonnene Gesamtalkaloidfraktion an Aluminiumoxyd, trennten aus den Voacorin-Fraktionen dieses Alkaloid ab und unterwarfen die in den Mutterlaugen enthaltenen Basen nach Abtrennung schwerlöslicher Hydrobromide einer 24-stufigen Craig-Verteilung zwischen Citronensäure-Phosphatpuffer vom pH 4,8 und Benzol/Äther 1:1. Aus den Fraktionen 5–14 der Verteilung kristallisierte Vobasin nach Anreiben mit Äther; die Ausbeute betrug 0,07–0,1%.

Vobasin $C_{21}H_{24}O_3N_2$ (ber. C 71,57; H 6,86; N 7,95; gef. C 71,26; H 6,90; N 7,88), aus Äther derbe Würfel, Smp. 111–113°C; $[\alpha]_D^{23}$-158,5° ($CHCl_3$, c = 1); −148,3° (MeOH, c = 1), enthält je eine OCH_3– (ber. OCH_3, 8,80; gef. 9,06), eine N–CH_3– (ber. CH_3 4,26; gef. 4,39) und eine C–CH_3-Gruppe (ber. CH_3 4,26; gef. 3,48). Bei der Mikrohydrierung mit PtO_2 in Äthanol wurden 2 Mol H_2 verbraucht. Das UV-Spektrum (λ_{max} 239,5 mμ; log ε 4,19 und 315 mμ, log ε 4,27) entspricht dem des 2-Phenylindols[8]. IR-Spektrum in CH_2Cl_2: Banden bei 2,91 (NH); 3,60 (N–CH_3?); 5,79 (C=O, Ester) und 6,07 μ; in Nujol: starke Doppelbande bei 13,45 und 13,58 μ (1,2-disubst. Phenyl).

Vobasin-Hydrochlorid $C_{21}H_{25}O_3N_2Cl$ (ber. C 64,84; H 6,48; N 7,21; Cl 9,12; gef. C 64,41; H 6,49; N 7,28; Cl 9,07): aus Methanol prismatische Blättchen, Smp. 245 bis 248°C (Zers.); $[\alpha]_D^{22}$–120,1° (MeOH, c = 1).

Vobasin-Methojodid $C_{22}H_{27}O_3N_2J$ (ber. C 53,45; H 5,51; N 5,67; gef. C 53,31; H 5,58; N 5,71): aus Methanol prismatische Stäbchen, Smp. 212–214°C (Zers.); $[\alpha]_D^{23}$–117,2° (MeOH, c = 1).

Die physikalischen Daten der Base und ihres Hydrochlorids schliessen eine Identität mit Voacafrin oder Voacafricin aus.

Die im Vobasin enthaltene OCH_3-Gruppe ist Teil einer Methylestergruppierung. Alkalische Verseifung führte zu einer Aminosäure $C_{20}H_{22}O_3N_2$ (ber. C. 70,98; H 6,55; N 8,28; gef. C 70,94; H 6,59; N 8,43), Smp. 290–292°C (Zers.), die mit Diazomethan *Isovobasin*, $C_{21}H_{24}O_3N_2$ (ber. C 71,57; H 6,86; N 7,95; OCH_3 (1) 8,80; gef. C 71,40; H 6,86; N 8,05; OCH_3 8,37); Smp. 175–178°C (Zers.); $[\alpha]_D^{22}$ − 191,3° ($CHCl_3$, c = 1), lieferte. Gewisse Parallelen zwischen Vobasin und der stärker basischen Molekülhälfte des Voacamins (N–CH_3-Gruppe[9], Umlagerung bei alkalischer Verseifung[10]) lassen vermuten, dass Vobasin einer monomeren biogenetischen Vorstufe des dimeren Voacamins nahesteht.

Ein weiteres Nebenalkaloid, für das wir den Namen *Voacryptin* vorschlagen, wurde nach 24stufiger Craig-Verteilung von Voacamin-Mutterlaugen zwischen Citronensäure-Phosphatpuffer vom pH 4,2 und Benzol/Äther 1:1 aus den Fraktionen 20–24 isoliert: aus Äther lange, verfilzende Nadeln, Smp. 175–176°C; $[\alpha]_D^{22}$ + 24,7° ($CHCl_3$, c = 1). Das UV-Spektrum (λ_{max} 224 mμ, log ε 4,46 und 285 mμ, log ε 3,98) entspricht dem des Voacangins. Summenformel ($C_{22}H_{26}O_4N_2$ ber. C 69,09, H 6,85; N 7,33; OCH_3 (2) 16,22; C–CH_3 (1) 3,93; gef. C 68,83; H 6,91; N 7,63; OCH_3 16,22; C–CH_3 3,21) und IR-Spektrum in CH_2Cl_2 (Banden bei 5,80 [C = O, Ester] und 5,83 μ [C = O, Keton]) weisen auf die Struktur eines Oxovoacangins.

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