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ENDOGENOUS GROWTH FACTORS AND FLOWER FORMATION IN WEDGWOOD IRIS BULBS

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ABSTRACT

1. The changes in growth substance compcsition of buds and scales of Wedgwood iris bulbs during flower formation were determined.
2. The scales contain several thermostable flower forming substances which, in the course of a low temperature treatment, are transported to the bud.
3. The flower forming substances have physiological and chemical properties of gibberellins.
4. Compounds with R_F 0.3, 0.45–0.50 and 0.75 in 80 % isopropanol successively accumulate in the bud and attain maximum concentrations at successive stages of differentiation of the floral primordium.
5. The significance and possible mode of action of the gibberellins in flower formation is discussed.

INTRODUCTION

Bulbs of Wedgwood Iris are vegetative when lifted. Induction and differentiation of flower primordia takes place during a 9 to 10 week's temperature treatment at 13° C. (HARTSEMA and LUYTEN, 1955).

Preceding reports have suggested the bulbs contain substances that are able to promote flower formation in excised apices of the same cultivar. These substances are found in buds and scales, in reproductive and vegetative state, before and during the flower inducing low temperature treatment (RODRIGUES PEREIRA, 1961, 1962). It was also suggested that these substances were related to or in the class of gibberellins.

These findings raised several interesting problems as to origin, nature and mode of action of these compounds. The relationship between scales and bud, the significance or direct effect of temperature treatment, etc.

In experiments to be discussed we have sought additional information on these questions. These studies show that in flower formation in Wedgwood iris several gibberellin-like compounds take part; these compounds are successively transported from scales to bud, depending on duration of the cold treatment and developmental stage of the primordium. Low temperature does not seem to induce production of flower forming substances but only to condition the growing point so that it may respond to them.

MATERIAL AND METHODS

1. *Culturing of isolated parts of iris bulbs*

Bulbs of Wedgwood iris, lifted about mid-August, were obtained commercially. In order to be assured of maximum flowering capacity, bulbs with an average circumference of 10½ to 11 cm were used. They were stored in a dark room that was kept at 26.5 ± 1° C and 60 to 70 % relative humidity.

Procedures for desinfecting bulbs and isolating and culture of buds and scales were as already described (RODRIGUES PEREIRA, 1962). For the investigations which follow two minor modifications were applied.

When single scales were planted on nutrient agar in culture-tubes they were not cut straight at the base but the level of contact with the medium was enlarged by making three to four triangular incisions of about 1 cm deep and 0.5 cm wide at the lower end. The teeth so-formed on the scales were pushed 1 cm deep into the agar.

Secondly, instead of Arnon's micronutrient solution (ARNON, 1938) the one composed by HELLER (1953) was used, in which $FeCl_3$ was replaced by an equivalent quantity of FeEDTA. Medium consisted of Knop's solution half strength to which were added 1 ml per liter of Heller's modified micronutrient solution, 4 % sucrose, and 0.9 % agar. Pyrex distilled water was used for all media; tap water for sterilizing procedures.

Medium was sterilized by autoclaving at 120° C for 20 minutes. When extracts or eluates of chromatograms were tested, they were separately sterilized by filtration through a millipore filter according to the method of Nitsch (NITSCH and NITSCH, 1957) and added to the autoclaved basic medium after the latter cooled down to approximately 40° C.

Every experimental series consisted of 24 test stem disks.

2. *Extraction of growth substances*

a. *For paper chromatographic analysis.* Buds and scales were separately dried by freeze-drying. Dried material was pulverized in a mortar and stored in a dessiccator over sulfuric acid at — 10° C.

Three hundred milligrams of this material were extracted three times with 15 ml cold methanol at 4° C. Each extraction lasted 30 minutes.

Extracts were combined and concentrated under reduced pressure at 25–30° C to about 0.15 ml;

b. *Extraction and purification of greater quantities of material.* For obtaining greater amounts of partly purified extracts reproductive bulbs were used, i.e. bulbs already subjected to the temperature induction treatment for 6 or 7 weeks.

Buds were dried and stored in the manner indicated in 2a. *Scales* were partly dried for several hours in a ventilated oven at 60° C and then stored at — 10° C awaiting further handling. Then,

15 gram's samples were extracted three times with 1 liter cold methanol at 4° C. The crude extract was concentrated in a cyclone-evaporator (HARADA, 1962) and the concentrate freeze-dried. The dried extract was pulverized in a mortar and re-extracted at 4° C, with 1 liter water for every 15 grams of material.

Insoluble particles in the water extract were removed by filtration and the filtrate was frozen dry. The residual material was again pulverized and the powder extracted three times with ethylacetate, about 250 ml per 10 grams of material.

For biological assays the solvent was removed under reduced pressure at 30° C and the residue redissolved in water.

This procedure was followed with minor variations for both buds and scales. In experiments reported below no further purifications were applied.

3. *Paperchromatography*

Separation was carried out at 25° C on Whatman 3MM paper strips that were 3 cm wide. The solvent was isopropanol: water, 80:20 (v/v). On each strip a quantity of methanolic extract equivalent to 100 mg dry material was applied.

4. *Biological assays*

a. *Avena mesocotyl test.* This test was applied in connection with the paper chromatographic separation of growth substances in crude methanol extracts. It was carried out according to the method of NITSCH (1956) as fully described by PILET (1960).

We used the hulless oats variety Torch, obtained through the courtesy of Mr. H. Gerrie of the University of Saskatchewan, Saskatoon, Saskatchewan, Canada, to whom our thanks are due.

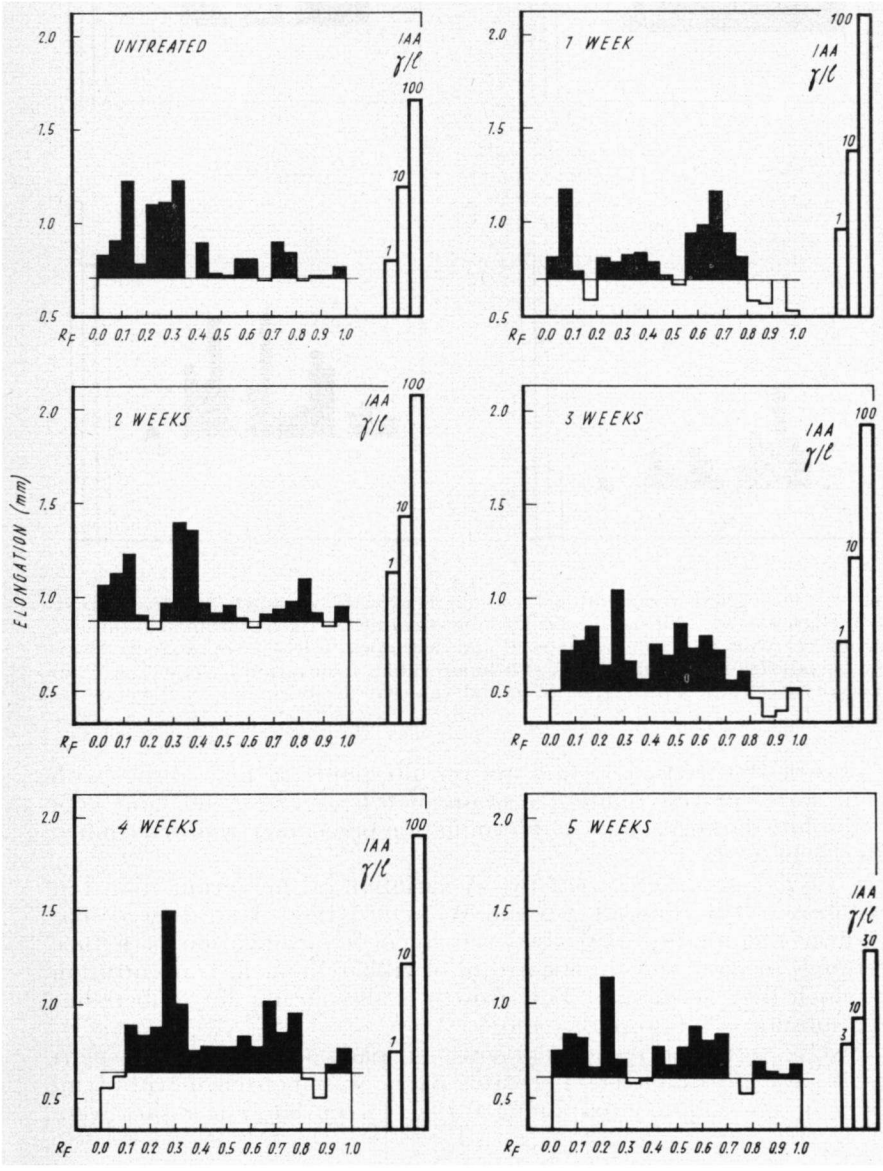
b. *Dwarf maize test.* The single gene mutant dwarf-5 of *Zea mays* L. was used. The mutants were obtained through the courtesy of Dr. B. O. Phinney to whom our thanks are due. Plants were grown in greenhouse flats containing garden soil at about 25° C. After 10 to 14 days a 0.1 ml aliquot solution, containing 0.1 % Tween 80, was placed in the axil of the first leaf with a 0.1 ml pipette. Solutions were replicated on 10 assay plants. A duplicate group of 10 plants were used as controls and received 0.1 ml 0.1 % Tween 80. After 10 to 14 days the first and second leaf sheaths were measured from node to level of ligule. The length of the leaf sheaths was compared to that of plants treated with solutions of 100 or 1000 μg gibberellic acid (GA) per liter.

c. *Dwarf pea test.* We used a dwarf mutant of *Pisum sativum* produced at the Laboratory for Horticulture of the Agricultural University, Wageningen and kindly put at our disposal by Prof. S. J. Wellensiek. It was established that this mutant reacts strongly on application of gibberellic acid. Thus it can be used as a fairly specific test object for gibberellin-like substances.

Seeds were sown in greenhouse flats in soil at 10 to 15° C. Fourteen days after sowing seedlings had two or three dark green or blue green

leaves. They were selected for uniformity and treated. A 0.1 ml aliquot of a solution of the plant extract or of increasing concentrations of GA in water containing 0.1 % Tween 80 was applied to the "heart" of the seedling. The plants were then kept at 25° C and after 14 days the shoot was measured from coleoptilar node to growing point.

d. *Lettuce seed germination test.* Seed germination in total darkness of *Lactuca sativa* cv. 'Grand Rapids' is specifically promoted by gibberellin-like substances (SKINNER *et al.*, 1958).



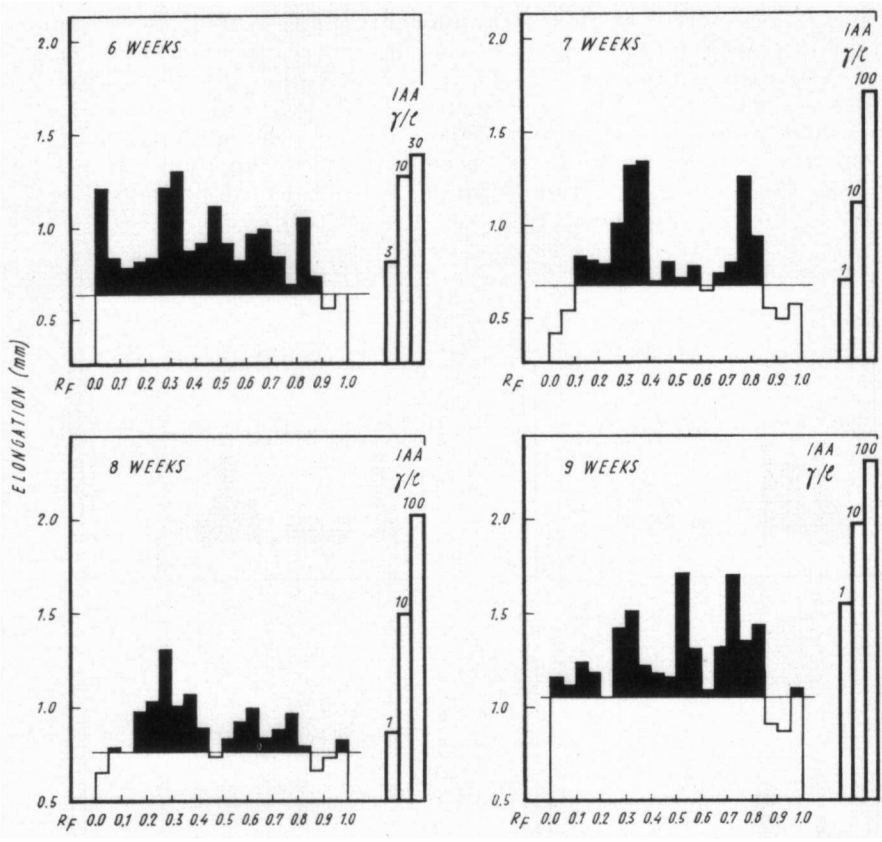


Fig. 1. Histograms representing variation in growth substance activity in buds of Wedgwood iris during flower forming treatment. Each chromatogram was carried out with 100 mg. dry material and developed in 80 % isopropanol. Abscis values: position on chromatogram. Ordinate values: elongation of segments of oat mesocotyls; means of 10 segments; initial value at the beginning of the test was 4 mm.

Lots of 100 seeds were laid out on filterpaper in petri dishes with 5 ml water or test solution containing 0.1 % Tween 80, and kept in absolute darkness at 25° C. Germination percentage was determined after 5 or 6 days.

e. *Lettuce seedling hypocotyl test.* A variation of the germination test was proposed by FRANKLAND and WAREING (1960) who showed that a linear relationship exists between hypocotyl elongation of lettuce seedlings in light and the logarithm of gibberellic acid concentration in which they germinate. This growth is also specific for gibberellin-like substances (THOMPSON, 1961).

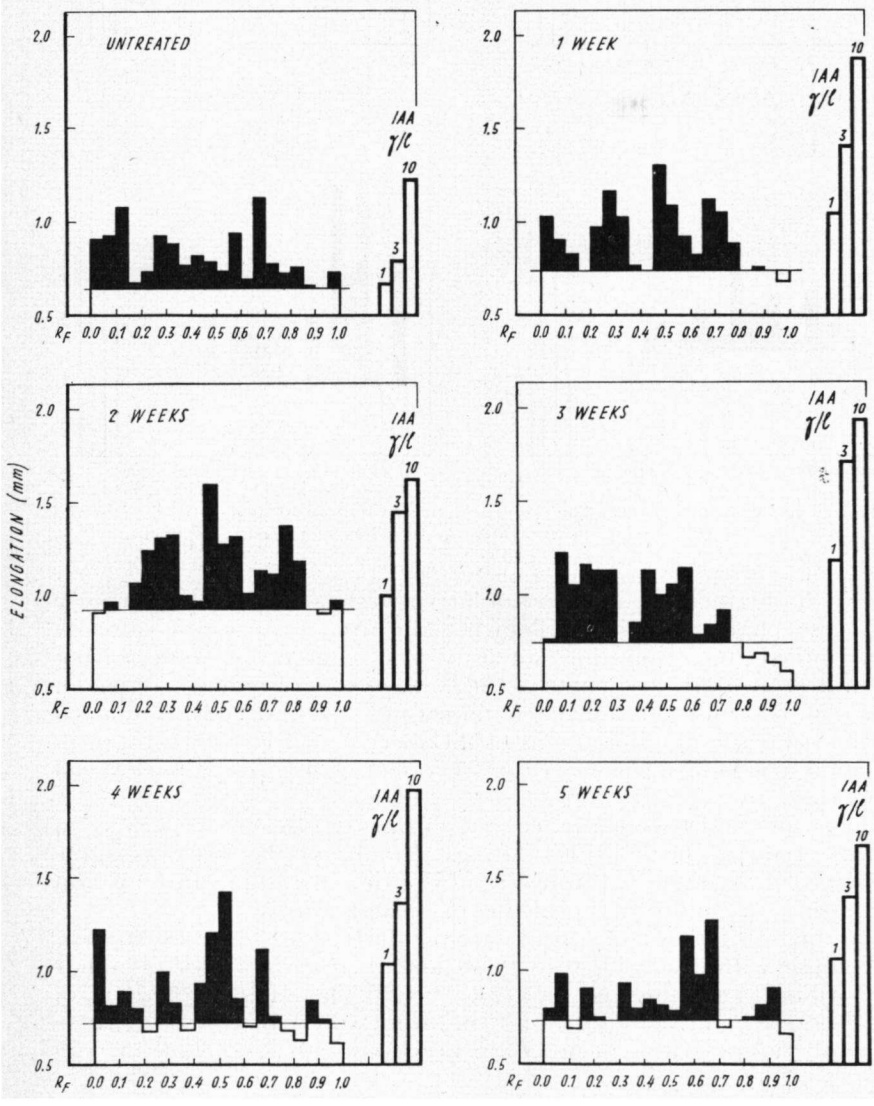
Twelve to fifteen seeds were sown on pads of filterpaper in petri dishes of 4 cm diameter. The filter paper was moistened with 1 ml water or test solution containing 0.1 % Tween 80. The dishes were

placed on the laboratory desk in natural daylight. When chromatograms were assayed in this way, the strips were cut into 2 cm sections which were placed below the filter pads in the petri dishes. After 5 or 6 days hypocotyls of 10 seedlings at random were measured to the nearest millimeter.

Results

1. *Changes in endogenous growth substances during flower induction and differentiation*

Prolonged experimental low temperature treatment causes continuous changes in the iris bulbs. Therefore, to be able to correlate



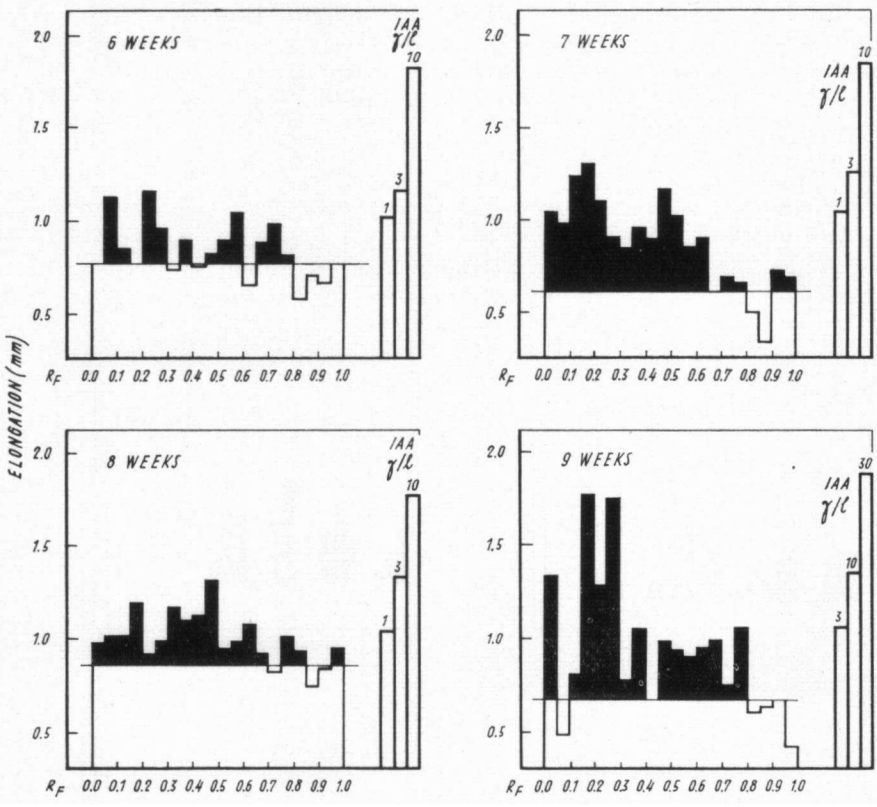


Fig. 2. Histograms representing variations in growth substance activity in scales of Wedgwood iris during flower forming treatment. Legends as in Fig. 1.

the data obtained in the course of later investigations, the time course of these changes must be known. This time course was already determined for respiration intensity and content of some carbohydrates (RODRIGUES PEREIRA, 1962). As we were now interested in the flower forming substances contained in scales and buds, we started with systematic determinations of the spectrum of growth substances in these parts during a 9 week's treatment at 13°, using the Avena mesocotyl test.

Determinations were carried out during two successive years, on bulbs of the 1960 and 1961 liftings. Results of the two lots agree rather well. In Figures 1 and 2 results of determinations in buds and scales of 1961 bulbs are presented as histograms.

In the histograms 5 or 6 peaks are regularly seen. The most striking are those with R_F 0.30, 0.45–0.50, and 0.70–0.75. Peaks of minor importance sometimes occur at R_F 0.1, 0.80 and 0.95. A zone of inhibitors is immediately behind the fromt; these inhibitors increase as temperature treatment proceeds. Fractions with R_F 0.30, 0.45–0.50

and 0.70–0.75 were studied in greater detail; they will henceforth be indicated as A, B, and C respectively. Fractions A and C are hardly ever missing and reach especially high values in the buds. In scales, fraction B is almost continually present in higher amounts than the other two, whereas in buds B is lowest and at times even seems to be entirely absent.

Figures 1 and 2 already suggest that developmental stage of flower primordium is correlated with, or at least is reflected in, the spectrum of growth substances. This idea was confirmed by using bulbs that had been activated by a prolonged storage at a rather high temperature, i.e. about 16–19° C. This lot of bulbs when received just showed the first beginning of tepal formation and thus were in stage III–IV, which is the stage reached in about 6 weeks of a regular 13° treatment. Growth substance activity of a methanolic bud extract of these bulbs is almost identical with that of a bud extract prepared after a storage

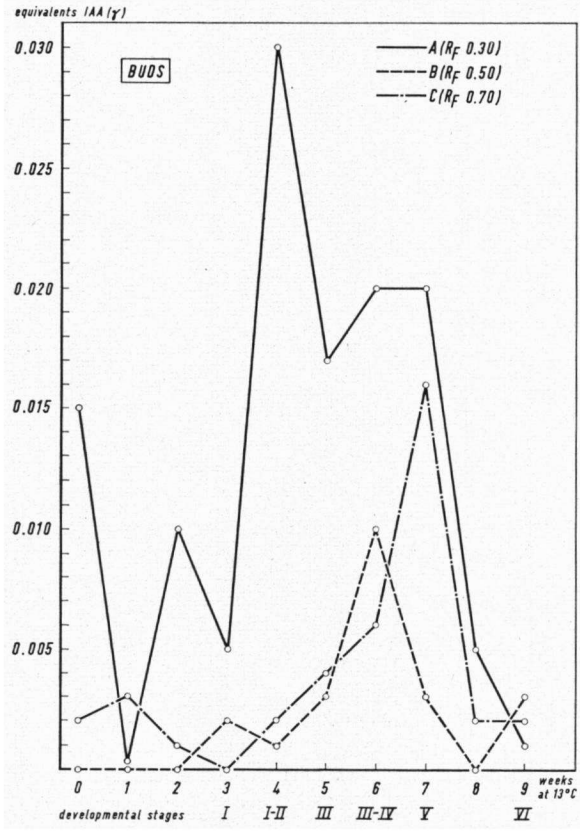


Fig. 3. Time course of contents of three different growth substances in buds of Wedgwood iris during flower forming treatment. Explanation in text.

period of 6 weeks at 13°. Distribution of growth substances in buds thus correlates with the stage of development of flower primordia and is independent of the way in which this stage is attained. That means that observing the stage of bud development should permit prediction of the growth factor present in highest amount.

The quantities of the different growth substances on the chromatograms may be expressed as equivalents indoleacetic acid (IAA). This was done in Figures 3 and 4. Of fractions A, B and C neither the maxima in bud extracts nor their minima in scale extracts do coincide. For each fraction, however, maximum in the bud and minimum in the scale is reached at about the same time. That is, in one organ the concentration goes up while in the other organ it goes down. In the buds the maximum of A occurs first, viz. at about the time of transition from vegetative to reproductive state, which happens after 4 to 5 weeks at 13° C. Compound B, although generally at a lower level, shows a greater equivalent IAA-value at time of formation of the first tepals, after 6 weeks at 13° C.

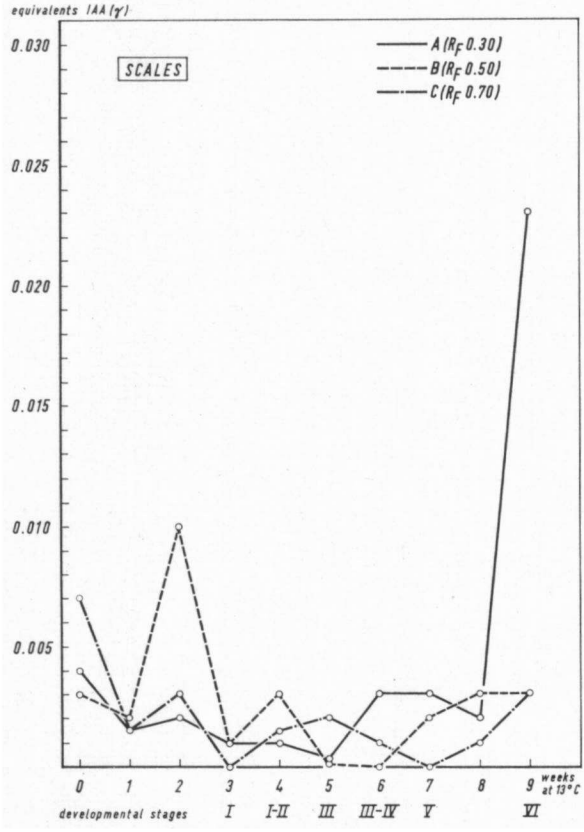


Fig. 4. Time course of contents of three different growth substances in scales of Wedgwood iris during flower forming treatment. Explanation in text.

Compound C has its maximum after 7 weeks at 13° C immediately before carpel formation.

All these maxima do correspond with minima in the—admittedly low—levels in the scales. It has to be kept in mind, however, that both series of chromatograms were prepared from 100 mg dry material. As determined earlier (RODRIGUES PEREIRA, 1962), total dry weight of the scales at the beginning of the cold treatment is about 8000 mg, and after 9 weeks it has decreased to about 6900 mg. In the same period dry weight of the bud increases from 185 to 800 mg. Thus it appears that absolute increase of each single growth substance in buds is not equal to absolute decrease in scales. This may be partly due to the fact that the method followed is perhaps not the most suitable for quantitative work. Neither is it possible to explain the decrease in the levels occurring in buds after the maxima have been reached as apparent and caused by dilution as a result of continuing growth. Binding or conversion to an inactive compound seems possible.

2. *The effect of scale diffusate and separate fractions of bud extract on flower formation*

Previous experiments established that the scales contain flower forming substances which by way of diffusion in nutrient agar influence excised stem disks (RODRIGUES PEREIRA, 1961, 1962). Scales were tested by either simultaneous incubation in the same tube as the stem disk, or they were taken from activated bulbs, i.e. bulbs exposed for 4 weeks to 13° C. In the latter case the scales were incubated for one week at 13° C on the agar, removed and replaced by non-activated test stem disks. These procedures do not differentiate between prior or subsequent formation of flower inducing substances in the scale. During the time the intact bulb is stored at 13° C interaction between scale and bud is obviously possible; also, when bud and scale are separated but incubated on the same agar tube, initial activation of the scale by the bud might take place and this might precede diffusion of flower forming substances from the scale. Therefore, scales taken from non-activated bulbs were incubated for four weeks on the nutrient medium; at the end of this period they were replaced by non- or slightly activated test stem disks. Controls consisted of scales taken from bulbs activated one week, as well as test stem disks implanted on media without scale diffusate. Table 1 shows that both activated and non-activated scales produced flower inducing substances which reached the stem disks. The experiment was replicated three times. We conclude from these results that either flower forming substances are already present in scales at the beginning of cold treatment or, that they are quickly formed from precursors at the onset of low temperature treatment without any intervention from the bud. In Fig. 4 curves of compounds A and C point more to the former possibility, that of compound B more to the latter.

Compounds A and C were eluted from the chromatogram, incorporated separately into the nutrient agar and tested on excised buds. Both compounds showed flower forming capacity (Table 2).

TABLE 1

Effect of previous incubation of detached scales on agar medium on flower formation in excised buds of Wedgwood iris.

Scales were replaced by test stem disks after three and four weeks respectively. Test stem disks were slightly activated before excision. Duration of incubation of stem disks 7 weeks. n_t = total number of test stem disks; n_r = number of stem disks found reprodyctive; score = average stage of flower formation in reproductive apices in equal units ranging from 0 to 14.

Treatment of scales before isolation	after isolation	n_t	n_r	score
1 week at 13° C.	3 weeks at 13° C.	21	8*	8
none	4 weeks at 13° C.	20	12**	7
control	without scales	24	2	6

* $P < 0.05$ ** $P < 0.001$

TABLE 2

Effects of fractions of bud extract on flower formation in excised buds of Wedgwood iris.

Test stem disks excised from activated bulbs. Extracts prepared from buds in stage III (staminal primodia formed). Amount of extract per tube equivalent to 5 buds. Other data as in Table 1.

Extract fraction	n_t	n_r	score
control	54	38	4
A (R_F 0.3) . . .	57	50*	6
C (R_F 0.70) . . .	56	48**	5
R_F 0.6	56	40	4

* $P < 0.02$; ** $P < 0.05$

At the time of extraction, buds were in stage III; Fig. 1 shows that at that stage content of A was greater than C. An eluate of R_F 0.6 separated from the same extract was tested as control. It did not promote flower formation, i.e. percent reproductive apices and average stage of flower formation in reproductive apices expressed in equal units ranging from 0 to 14 (score) were equal to those on the control medium. Although A and C reach their maximum concentration in the bud at different times during differentiation of floral primordium (Fig. 4), in their over-all effect on flower formation no difference in mode of action could be distinguished.

Neither compound B nor growth substances of the scales were separately tested on excised stem disks.

3. *Dose-response curve of bud extract*

Considering that a stimulating quantity of bud extract was equivalent to one bud per test stem disk (RODRIGUES PEREIRA, 1961) different concentrations of bud extract were added to the medium.

Test stem disks were implanted in tubes on media containing growth substances from the methanol extracts of 0.1, 0.3, 1.0, 3.0, or 10.0 buds. Experiments were replicated three times; extracts were always prepared from reproductive buds in stage III–IV. Table 3, results of one experiment with non-activated test stem disks, the effect is almost maximum at a ratio of one bud equivalent per stem disk.

TABLE 3

Effect of increasing concentrations of bud extract on flower formation in excised buds of Wedgwood iris.

Bud extract made of buds in stage III–IV (score 6). Test stem disks not activated. Duration of experiment 6 weeks. Other data as in Table 1.

extract concentr. as bud equiv./ stem disk	n_t	n_r	score
0	24	0	—
0.1	22	6	4
0.3	23	3	3
1.0	24	8*	4
3.0	21	5	6
10.0	20	8*	6

* $P < 0.01$

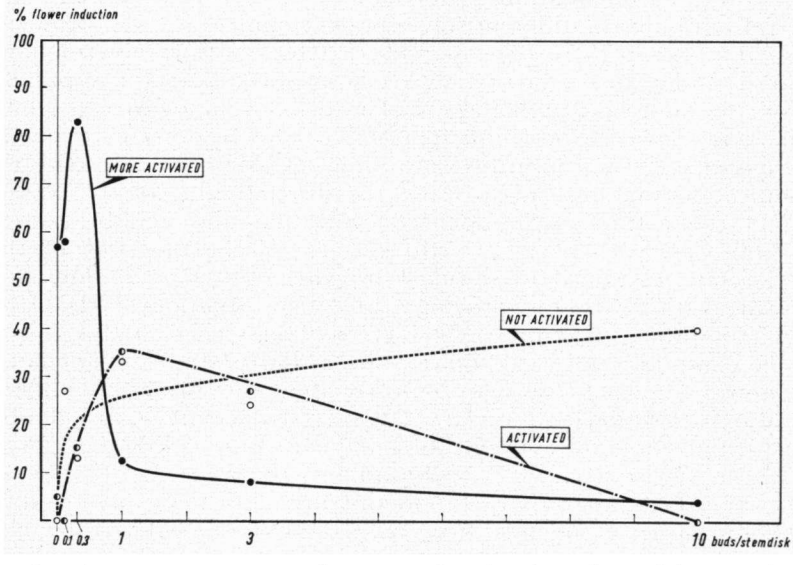


Fig. 5. Dose-response curves of extract of buds of Wedgwood iris on flower formation in excised buds. Extract made of buds in stage III–IV. Test stem disks were in three states of activation: non, slightly and more advanced. Observations made after 6 weeks.

Higher concentrations only slightly improve results. In the other two experiments, the maximum lay also at one bud equivalent per stem disk, but at higher concentrations the promotive effect of the extract decreased (Fig. 5). The three experiments differed in the rate of activation of test stem disks.

4. *Possible nature of the flower inducing substances*

In earlier publications (RODRIGUES PEREIRA, 1961, 1962) we already advanced the hypothesis that the flower inducing growth substances in bulbs of Wedgwood iris may be gibberellin-like. By means of several physico-chemical and biological test methods, reported to be more or less specific for gibberellins, we have tested this hypothesis. *Colour reactions* included spraying chromatograms with an aqueous solution of 0.5 % $KMnO_4$ and with a 5 % methanolic solution of sulfuric acid. *Biological assays* included Phinney's dwarf maize test, a dwarf pea test, a lettuce seed germination test and a lettuce seedling hypocotyl test.

For colour reactions mostly chromatograms of crude methanolic extracts were used. These crude extracts, however, proved to be toxic for the test plants in biological assays. Therefore, they were further purified as described in 2b of "Methods". By this procedure not all toxins were removed but their concentration was sufficiently lowered to enable use with test plants.

1. *Colour reactions.* After spraying with a 0.5 % aqueous solution of $KMnO_4$, chromatograms of methanolic extracts of buds and scales taken from bulbs in different stages of development showed brownish spots at R_F 0.3, 0.45–0.50, 0.65 and 0.75–0.80. Spots were particularly clear in those areas where the Avena mesocotyl test of a parallel chromatogram was at a high peak. Parallel chromatograms of gibberellic acid showed a brownish spot at R_F 0.45. In other experiments extracts of scales were purified according to "Methods" 2b, with the modification that instead of using the dried residue of the watery extract the extract itself, after concentration and acidification to pH 3.0 was extracted with ethylacetate. Chromatograms of this ethylacetate fraction showed a dark brown spot at R_F 0.75 only after spraying with $KMnO_4$. Spraying with a 5 % solution of H_2SO_4 in methanol produced strong yellow-green fluorescence at 254 mμ at R_F 0.65 and 0.75 and weak fluorescence at R_F 0.2 and 0.3. The latter reaction is known to be more sensitive than the former. Acidifying the watery fraction and extraction with ethylacetate had a double effect. It increased the concentration of the fraction with R_F 0.65–0.75, and decreased concentration of inhibitor.

2. *Dwarf maize test.* Extracts of both buds and scales caused elongation of sheaths of the first two leaves of Phinney's dwarf-5 maize mutant, just as occurs specifically with gibberellins. A typical experiment is represented in Table 4.

3. *Dwarf pea test.* The dwarf mutant of *Pisum sativum* proved very sensitive to gibberellic acid and only slightly so to indoleacetic acid;

this is also the case with dwarf variety Meteor (BRIAN and HEMMING, 1958; RADLEY, 1958). Both bud and scale extracts were tested. Results of these assays are presented in Tables 5 and 6 and in Fig. 6. The effect of bud extract and gibberellic acid solution was already evident three days after application of 0.1 ml; the colour of treated young leaves was lighter than of controls. Differences in colour are more marked still in Fig. 6, photographed 12 days after application of 0.1 ml of test solution.

TABLE 4

Effect of an extract of scales of iris bulbs on elongation of leaf sheaths of dwarf-5 mutant of maize.

0.1 ml H_2O or test solution, containing 0.1 % Tween 80, was applied to the axil of the first leaf; seedlings were measured after 14 days. Each treatment consisted of 10 plants; 0.1 ml extract contained growth substances of scales from 1.25 bulbs.

treatment	length in cm		
	1st sheath	2nd sheath	total
H_2O	1.42 ± 0.14	2.42 ± 0.18	3.84 ± 0.23
GA 1 µg/ml . . .	1.89 ± 0.09	4.28 ± 0.16	6.19 ± 0.18
extract	1.67 ± 0.14	2.79 ± 0.27	4.44 ± 0.30

TABLE 5

Effect of gibberellic acid, indoleacetic acid and extract of scales of Wedgwood iris on growth of dwarf mutant of pea.

0.1 ml test solution in 0.1 % Tween 80 was brought in the "heart" of the seedlings. Seedlings were 14 days old at the time of the experiment, and their length, from soil to growing point, was measured after another 14 days. Each treatment consisted of 10 plants; 0.1 ml scale extract contained the active principle of the scales of 0.5 bulb.

treatment	length in cm
H_2O	3.68 ± 0.30
GA 0.001 µg/ml	4.14 ± 0.13
0.01 µg/ml	5.38 ± 0.22
0.1 µg/ml	8.75 ± 0.30
scale extract	4.15 ± 0.12

TABLE 6

Effect of the extract of buds of Wedgwood iris on growth of dwarf mutant of pea.

On 5 consecutive days 0.1 ml of test solution was brought in the "heart" of the seedling; 0.1 ml extract contained the active principle of 1.5 reproductive buds. Other data as in Table 5.

treatment	length in cm
H_2O	3.47 ± 0.4
GA 1 µg/ml	8.80 ± 0.50
bud extract	5.21 ± 0.14

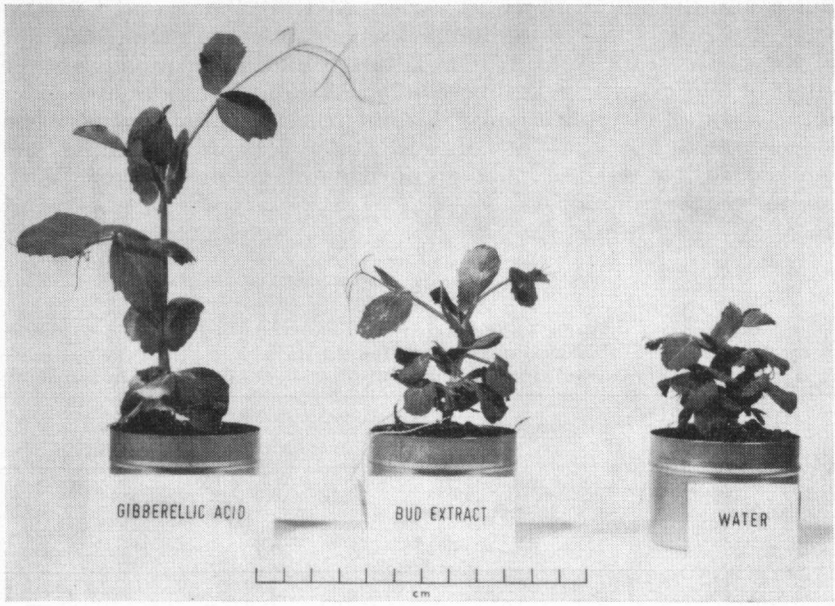


Fig. 6. Growth promoting effect of extract of buds of Wedgwood iris on dwarf pea seedlings. Gibberellic acid solution contained 1 μg/ml 0.1 % Tween 80. Bud extract contained active principles of 20 buds per ml. 0.1 % Tween 80. When two weeks old seedlings were treated with 0.1 ml on 5 consecutive days. The photograph was taken 12 days after the first application.

TABLE 7

Effect of a partly purified extract of buds of iris bulbs on the hypocotyl growth of seedlings of Lactuca sativa cv. 'Grand Rapids'

Five cm petri dishes plus 1 ml H_2O or test solution and 10 to 15 seeds. Germination in the light at 20°. After 5 days hypocotyls of 10 seedlings were measured to the nearest millimeter.

treatment	length of hypocotyl in mm
H_2O	5.7 ± 0.4
gibberellic acid	
0.01 μg/ml	7.0 ± 0.5
0.1	9.9 ± 0.4
1	14.7 ± 0.9
bud extract	
0.2 bud/ml	5.9 ± 0.5
0.5	7.3 ± 0.6
1	7.1 ± 0.4
2	5.5 ± 0.6
4	2.5 ± 0.5

4. *Lettuce seed germination test.* Germination of lettuceseed cv. 'Grand Rapids' in absolute darkness was promoted by bud and scale extracts. Germination at increasing concentrations showed a typical optimum curve.

5. *Lettuce seedling hypocotyl test.* Bud and scale extracts had a promoting effect on growth of hypocotyl of lettuce seedlings. The extracts were applied in several concentrations. A typical experiment, carried out with bud extract, is presented in Table 7. At higher concentrations inhibition of hypocotyl growth occurs. The same result was found with scale extract still further purified over Dowex 2.

Discussion

These investigations clearly established at least the following three points.

First: it was shown by chromatograms of methanol extracts that all the different growth substances that are detected over a 9 week's cold treatment are already present in untreated bulbs. However, amounts in buds are insufficient to induce flower formation, even at the optimum temperature, whereas in scales they are present in amounts that are sufficient to induce flower formation in excised buds. It follows then that most probably they are not formed during or as a result of the cold treatment, but are formed in summer, during development and growth of the bulb. If this is correct then the effect of cold treatment may be to activate transport of the flower forming substances from scales to bud and render the apex fit to respond to them. A similar effect has been established in the case of some carbohydrates (Rodrigues Pereira, 1962).

Secondly: different growth substances, distinguished by R_F's in 80 % isopropanol do not accumulate in the bud at the same rate. The sequence in which they accumulate is constant and correlated with development of flower primordium. It may be that each regulates a special stage of flower bud differentiation, following activation of the growing point. It was, however, established that both compound A (R_F 0.3) and C (R_F 0.70) that reach a maximum concentration in the bud with an interval of three weeks, promote flower formation in excised buds if added to nutrient medium.

Thirdly: by means of specific biological assay methods and, admittedly less specific, colour reactions, it was demonstrated that at least three fractions of the bulb extracts are physiologically related to the gibberellins. Compound C clearly has an acid character, A and B probably have not.

Increasing concentrations of crude and partially purified extracts were assayed on test stem disks of iris in order to test their flower forming capacity and on lettuce seed in order to test gibberellin-like activity (Fig. 5, Table 7). With one exception the resulting dose-response curves looked like normal optimum curves, as are known for pure IAA, boron, magnesium etc. Yet there are many arguments

against this interpretation and for the assumption that the form of the curve is due to activity of inhibitors.

The presence of growth inhibitors in crude bud and scale extracts is established by the *Avena* mesocotyl test (Figs. 1 and 2). Amount of inhibitors in the bud increases with duration of low temperature treatment. The experiment with increasing concentrations of crude bud extract tested on non-activated buds did not show inhibition of flower formation even at the highest concentration (Table 3). In the experiment reported in Table 2 test stem disks were somewhat activated as is shown by the high percentage of reproductive buds in the control (70 %). Compounds A and C, eluted from chromatograms and thus probably freed from inhibitors, were applied in a concentration of 5 bud equivalents per stem disk and still they significantly promoted flower formation. Up to a concentration of 100 μg/ml gibberellic acid does not inhibit flower formation in excised stem disks of Wedgwood iris (RODRIGUES PEREIRA, 1962). JOUANNEAU–SKAKOUN (1962), studying effect of high concentrations of gibberellic acid on growth of a dwarf pea variety never observed inhibition or even decreased growth promotion up to concentrations of 102.4 μg per plant. Inhibitive effects of high amounts of gibberellic acid are seldom reported. It is only von BOGUSLAWSKI (1962) who observed a decreased over-all yield after application of elevated doses of gibberellic acid (up to 10 or 40 μg per plant) to field crops. From all these experimental data we conclude it to be highly improbable that the form of the dose-response curves of our bud and scale extracts is due to mode of action of gibberellin-like substances. On the other hand the decrease in promotion both of flower formation, seed germination and hypocotyl growth could very well be due to inhibitor activity. According to the concept drawn by WELLENSIEK (1962) flower bud formation depends on the ratio of promoting and inhibiting substances.

We may now discuss in greater detail how these gibberellin-like substances work in promoting flower formation. We already demonstrated that substances diffusing out of scales into nutrient agar (cf. Table 1) are heat stable and that the inhibition of flower formation by high temperatures is not caused by destruction of the essential gibberellin-like substances, but must be of a complex metabolic nature (RODRIGUES PEREIRA, unpublished).

The number of plant species in which gibberellin-like substances are demonstrated with certainty has steadily increased (KNAPP, 1963). We are especially interested in those investigations which relate these substances with induction of flowering.

HARADA (1962) isolated compounds from *Althaea rosea*, *Rudbeckia speciosa* and *Chrysanthemum morifolium* cv. 'Shuokan' that induce shooting and flowering in his assay plants. These compounds are very much alike in physiological respect and possess many of the specific physiological properties of gibberellins, but their ultra-violet and infra-red spectra indicate that chemically they are not of this class. Our compound C, which has the same R_F in 80 % isopropanol as Harada's substance E is probably more like gibberellins in chemical

respect. LANG, SANDOVAL and BEDRI (1957) caused shooting and flowering in *Hyoscyamus* and *Samolus* with a gibberellin-like substance isolated from *Echinocystis macrocarpa*.

By grafting experiments ZEEVAART and LANG (1962) and HARADA (1962) arrived at almost identical answers to the question of mode of action of gibberellic acid in flower induction. ZEEVAART and LANG showed that in *Bryophyllum daigremontianum*, a long-short-day plant, GA is able to replace the transition from long day to short day but not the short day itself. A non-induced scion, grafted onto a stock that had been treated with GA, flowered in short-day. HARADA showed that the short-day cultivar Honeysweet of *Chrysanthemum morifolium* cannot be induced to flower by gibberellic acid but this compound can replace the cold treatment in the cold requiring cultivar "Shuokan". A non-induced "Honeysweet" grafted onto a "Shuokan" stock that had been induced by GA flowered in long days. In both cases it is conceivable that GA initiates or regulates production of the floral stimulus in the non-induced plant or scion. Bonner and co-workers (SALISBURY and BONNER, 1960; BONNER and ZEEVAART, 1962; ZEEVAART, 1962a and b) demonstrated that the response of the growing point to flowering hormone in *Pharbitis nil* and *Xanthium* is linked with DNA- and RNA-synthesis respectively. Similar conclusions were arrived at by HESS (1959) in the course of his experiments on *Sphaerocarpus wendlandii*. In preliminary experiments we were able to confirm that antimetabolites such as 2-thiouracil, 5-fluorouracil and 8-azaguanine inhibit flower formation in excised buds of Wedgwood iris. Inhibition by 2-thiouracil was reversed by increasing concentrations of orotic acid and thymidine, but also by gibberellic acid. However, as an antidote against the inhibition of DNA-synthesis by 5-fluorouracil in the apex of *Pharbitis nil*, gibberellic acid proved quite uneffective (ZEEVAART, 1962b).

It is known that in germinating seeds gibberellic acid stimulates the activation of several enzymes such as amylase (PALEG, 1960a and b), protease, katalase, phosphatase and cellulase (DAHLSTRØM and SFAT, 1961). These authors conclude that most probably the stimulation occurs at the level of enzyme synthesis. Each enzyme would be produced under the influence of a definite gibberellin-like substance, derived from gibberellic acid. Similar conclusions might be drawn from the work of NAPP–ZINN (1963) with multiple alleles of *Arabidopsis thaliana*. SAHAI SRIVASTAVA and MEREDITH (1962) showed that gibberellic acid partly reversed inhibition of enzyme synthesis by chloramphenicol. Experiments in progress in our laboratory indicate that growth inhibition of the hypocotyl of seedlings of *Lactuva sativa* cv. 'Grand Rapids' in the light by chloramphenicol and 2-thiouracil is partly reversed by increasing concentrations of gibberellic acid. It appears, however, from the respective dose-response curves, that not the same metabolic reaction is concerned in the two cases.

Summarizing, we can say that in many plants, i.e. *Xanthium*, *Pharbitis nil*, *Chrysanthemum morifolium* etc. at least three classes of

compounds are required for flower formation, viz. gibberellins, a flowering hormone of yet unknown nature, and specific nucleic acids. As for iris, here the second link seems to be absent. In this case an identity of flowering hormone and gibberellin-like substances might even be postulated.

The well-known fact (BLAAUW, 1934) that bulbs below a certain minimum size do not produce flower primordia but only foliage leaves, may well be due to a shortage of gibberellins.

When, at the onset of the cold treatment, the block on the metabolism is released and cell divisions are induced in the apex, the several gibberellin-like substances may regulate the differentiation of different parts of the flower primordium via synthesis of specific nucleic acids.

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