Table 2. Amount of compound II and IPA isolated from sRNA.

Source of sRNA	sRNA taken (g)	Yields	
		Compound II (mg)	IPA (mg)
Sweet corn kernels	10.05	1.9	0
Garden peas	5.24	0.27	0.13
Spinach	2.42	.25	.34

than those in the spectrum of compound II.

The sRNA was extracted from 45 kg of frozen spinach and 45 kg of frozen garden peas by a procedure identical with that used for the corn (Table 2). These samples were hydrolyzed and fractionated on partition columns, and elution patterns similar to the one shown in Fig. 1 were obtained.

Fraction 1 of the pea and of spinach sRNA eluates was chromatographed in solvent E, and pure IPA was obtained in each case. The isolated nucleoside was identified by its ultraviolet spectrum and cochromatography with a synthetic sample (*2*) of IPA. Compound II was isolated from the fraction 2 of each sample of sRNA and identified by ultraviolet spectroscopy and by chromatographic comparison with the nuceloside obtained from corn sRNA. The yields are recorded in Table 2.

On the basis of the procedure used and the sedimentation coefficients obtained, the RNA samples appear to consist, principally, of sRNA. These samples, however, could contain some degraded ribosomal RNA, and if one makes the assumption that compound II occurs only in the sRNA then the values recorded in Table 1 may be lower than actual amounts of this nucleoside in the sRNA. In mammalian tissue, IPA occurs only in the sRNA fraction (*3*).

The evidence for assignment of structure II to the nucleoside isolated from plant sRNA can be summarized as follows. (i) The ultraviolet absorption spectra are those of an N^6-(alkyl substituted)adenosine. (ii) The mass spectrum conforms to structure II. (iii) The NMR spectrum shows that compound II contains a methyl group attached to a vinyl carbon which does not have an attached proton. (iv) The specific optical rotation of II is similar to that of IPA (*3*) and is identical with that of a synthetic sample of compound III measured in the same instrument under the identical conditions. Therefore II is the β anomer. (v) In a comparison with ribosylzeatin, III, the melting point of II is 206°C compared to 181°C for III (*10*). On paper chromatography, compound II migrates slightly faster than III in several systems (Table 1).

On the basis of these data we conclude that this nucleoside isolated from plant sRNA consists of a 4-hydroxy-3-methylbut-2-enly side chain attached to the N^6 position of adenosine. Since the isolated nucleoside is not identical with the synthetic compound III we have assigned the *cis* configuration, structure II, to this component of sRNA.

Letham *et al.* (*12*) isolated from an extract of immature corn kernels a purine base identified as 6-(*trans*-4-hydroxy-3-methylbut-2-enylamino)purine, zeatin (*10*) (the base of structure III). This compound also has been obtained as its nucleoside and nucleotide (*14*), and the evidence suggests that these compounds exist in the corn kernels in an unbound form.

Compound II represents the second isoprenoid adenosine to be identified in sRNA. A third such derivative has been detected in the sRNA of yeast and mammalin tissue (*15*). These findings indicate that a series of isoprenoid nucleosides occurs in the sRNA although all members of this series do not necessarily occur in the sRNA of a given species.

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References and Notes

1. Abreviations used are as follows: sRNA, soluble RNA; IPA, N^6-(Δ^2-isopentenyl)adenosine; DEAE, diethylaminoethyl; OD, optical density; *M*, molecular ion; *m/e*, mass-to-charge ratio; NMR, nuclear magnetic resonance spectroscopy; δ, parts per million; *c*, g/100 ml.
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Apple Fruit-Set: Evidence for a Specific Role of Seeds

Abstract. *An extract of immature Wealthy apple seeds, containing substances with gibberellin activity, was applied to unpollinated blossoms of the same variety, resulting in the production of mature seedless fruits. The implications with respect to the normal process of fruit-set are discussed.*

Since the work of Gustafson (*1*), parthenocarpy has been induced in many species with synthetic auxins. However, attempts to induce parthenocarpy in apple with auxins were relatively unsuccessful (*2*). Success awaited the discovery and use of the gibberellins, Luckwill (*3*) being the first to note the effects of gibberellic acid (GA_3) in inducing parthenocarpy in apples. Endogenous gibberellins have been found in apple seeds (*4*), and two of these gibberellins have been identified as GA_4 and GA_7 (*5*). My study was initiated to determine whether substances with gibberellin activity, extracted from immature apple seeds, were capable of inducing fruit-set in unpollinated apple flowers.

The Wealthy variety was chosen as experimental material because of its parthenocarpic tendency (*6*) and the marked response of unpollinated Wealthy flowers to gibberellins (*6*, *7*). Fruits were picked from mature trees 1–9 July 1965 (6 to 7 weeks after full bloom and shortly after cessation of the June drop). The diameter of the fruit at this time was 30 to 35 mm, the length of the seed was 8.8 mm,

Table 1. Effects of KGA_3 or apple-seed extract (fraction 1) in lanolin pastes upon fruit-set, mean number of seeds per fruit, and mean diameter of fruit of unpollinated Wealthy apple flowers treated 26 May 1966. Fruit diameter was measured on 2 September. Branches bearing unpollinated flowers were scored 8 June, prior to the June drop, to limit abscission of seedless fruits. Open-pollinated flowers were borne on a separate branch which was not scored.

Treatment	Flowers treated (No.)	Set (%)		Seeds (No.)	Diameter (mm)
		8 June	5 September		
	Unpollinated flowers				
None	18	6	6	0	61
Paste alone	10	0	0	—	—
$5 \times 10^{-6}M$ KGA_3 in paste	16	0	0	—	—
$5 \times 10^{-5}M$ KGA_3 in paste	14	29	21	0	55
$5 \times 10^{-3}M$ KGA_3 in paste	15	73	60	0	57
Fraction 1 paste (1 kg-eq/g)	16	82	69	0	59
	Open-pollinated flowers				
	50	84	16	5.6	66

and the length of the embryo was 3.6 mm. The fruits were stored at 2°C; the seeds were removed within 32 hours of the time of picking, frozen on dry ice after removal from the fruits, and macerated in absolute ethanol. The slurry was left overnight at 5°C and then centrifuged; the supernatant was filtered through paper and evaporated to dryness (crude extract).

Substances with gibberellin activity were concentrated by dissolving the crude extract in distilled water, acidifying it to *p*H 3.0 with 1*N* formic acid, and extracting this aqueous solution with four equal volumes of ethyl acetate. The ethyl acetate was evaporated to dryness under vacuum, and the residue was dissolved in warm water and left overnight at 5°C to precipitate the phenolic glycoside phlorizin (*8*). The supernatant was evaporated to dryness and chromatographed on silica-gel columns (22 by 140 mm), 200 to 350 gram equivalents (g-eq) of extract being applied to each of five columns. Increasing concentrations of ethyl acetate in hexane were used for elution (*9*). Successive 100-ml volumes of each of the following concentrations were used: 0.5, 5, 10, 20, 30, 40, 50, 60, 70, and 100 percent ethyl acetate in hexane. Portions of each eluate were tested for gibberellin activity by lettuce-hypocotyl assay (*5, 10*). There was major activity in the 20, 30, and 40 percent eluates; these eluates from all batches of seeds (total of 1600 g) were combined for subsequent use (fraction 1).

The activities of the crude extract and of fraction 1 in the lettuce-hypocotyl test are compared with that of GA_3 in Fig. 1. The activity of fraction 1 was about one-tenth that of the crude extract (about 0.6 and 5.0 μg of GA_3 equivalents per gram fresh weight, respectively). Losses in purification are probably responsible for the major part of this difference; however, the steeper dosage-response curve of the crude extract compared to that of fraction 1 may indicate a synergism between gibberellin-like substances and other components removed in fractionation. Fraction 1 (1200 g-eq) was mixed with 1.2 g of a mixture of warm lanolin and Tween 20 (4:1) to give a concentration of 1 kilogram equivalent (kg-eq) of seeds per gram of paste (about $1.7 \times 10^{-3}M$ GA_3 eq). Similar pastes were prepared containing the potassium salt of GA_3 (KGA_3) at 0, 5×10^{-6}, 5×10^{-5}, and $5 \times 10^{-3}M$.

Three branches about 1 m long and 2½ to 5 cm in diameter at the base were selected from two mature Wealthy trees. On 21 May 1966, the blossom clusters were thinned to leave 15 to 20 cm between clusters, the unopened (balloon stage) flowers were reduced to two per cluster, and the styles were severed to prevent pollination. At full bloom (26 May) the remnants of petals, styles, and stamens were removed with a razor, and the lanolin pastes were applied to the cut surfaces and adjacent sepals, each paste being applied to several flowers on each branch. Fruit-set was evaluated on 8 June, and the branches were scored with a knife to limit abscission during the June drop (*6*). Open-pollinated flowers were thinned and allowed to develop on a separate branch.

All fruits which developed from unpollinated blossoms were seedless (Table 1). One fruit developed from a control blossom that was not treated with paste, as expected from previous experiments (*6*). However, treatment with the control paste prevented vegetative parthenocarpy (*11*). The minimum concentration of KGA_3 required to induce parthenocarpy was between 5×10^{-6} and $5 \times 10^{-5}M$. Both KGA_3 ($5 \times 10^{-3}M$) and fraction 1 induced a high percentage of the blossoms to develop into mature seedless fruits that were only slightly smaller than seeded fruits (Fig. 2).

Several investigators reported the production of parthenocarpic fruits in herbaceous plants after treatment with extracts of plant tissues (*12*), the tomato ovary being especially responsive to such treatment. To my knowledge, however, this is the first report of induction of parthenocarpy in a fruit with an extract prepared from the same species.

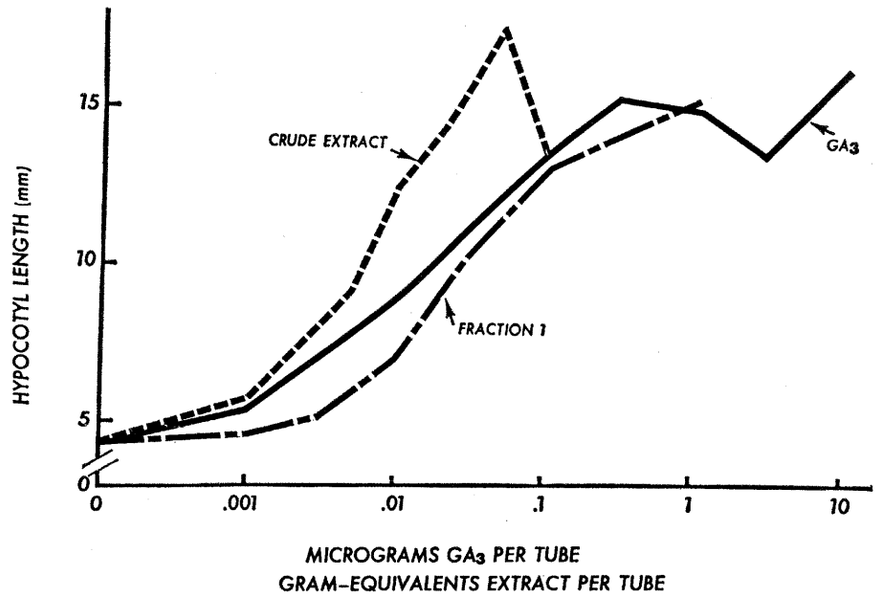

Fig. 1. Activities of crude extract and of partially purified extract (fraction 1) of Wealthy apple seeds in comparison with that of GA_3 in the lettuce-hypocotyl bioassay. Each point is the mean for two replicate tubes of five hypocotyls each.

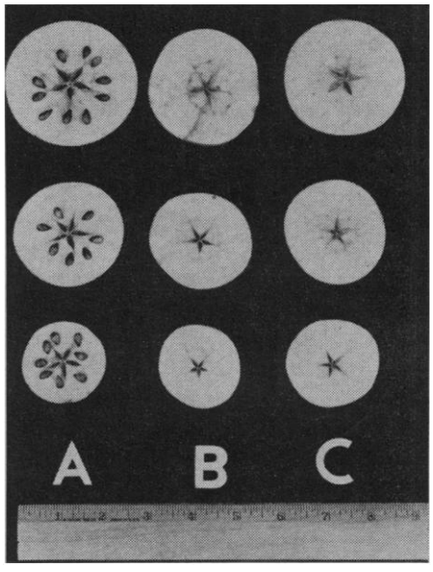

Fig. 2. Seeded and parthenocarpic Wealthy apple fruits. (A) Seeded fruits from open-pollinated flowers, with seeds removed from locules and placed on cut surface of fruit; (B) seedless fruits produced by treating unpollinated flowers with lanolin paste containing KGA_3 ($5 \times 10^{-3}M$); (C) seedless fruits produced by treating unpollinated flowers with lanolin paste containing apple-seed extract (1 kg-eq of fraction 1 per gram of paste). Paste applied 26 May; fruits photographed 7 September 1966.

The source of the extract (immature seeds) and its high gibberellin-like activity provide strong support for the hypothesis that gibberellins produced in the ovule after fertilization are responsible for fruit-set in the apple.

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Heat Inactivation of the Relaxing Site of Actomyosin: Prevention and Reversal with Dithiothreitol

Abstract. *Adenosine triphosphate and magnesium (MgATP) inhibit contraction by binding to a specific relaxing site on natural actomyosin gel. This inhibitory control site is distinct from the active sites where MgATP causes contraction. In high concentrations of MgATP, calcium triggers contraction by releasing the protein from substrate inhibition, allowing the contractile reactions to occur. Heating the protein for 5 minutes at 43°C selectively inactivates the relaxing site. After this treatment, actomyosin with MgATP contracts as well without calcium as with it. That this effect of heat is prevented and reversed by dithiothreitol (an agent that reduces disulfide bonds) indicates that the structure of the relaxing site depends on certain labile sulfhydryl groups, which may be those of tropomyosin. When these are oxidized to disulfide bonds, the site loses its activity; when the disulfide bonds are reduced, the site regains its activity.*

It is now generally believed that muscular contraction is controlled by changes in the amount of calcium available to the contractile filaments (*1*). Even though adenosine triphosphate and magnesium (MgATP), which cause contraction, are always present in high concentration (about 0.005*M*), the filaments cannot contract so long as the concentration of calcium in the sarcoplasm is held below some required level (about $10^{-7}M$). The sarcoplasmic reticulum maintains this relaxed condition by actively pumping calcium out of the sarcoplasm (*2*). Activation of the muscle allows the calcium concentration in the sarcoplasm to rise; calcium then binds to the filaments, and this binding causes them to contract.

Natural actomyosin is extracted directly from muscle; it contains tropomyosin and certain other proteins in addition to the main constituents—actin and myosin. Like the contractile filaments in muscle, such a gel in a high concentration of MgATP cannot contract or hydrolyze ATP without a trace amount of calcium (*3*). Without this calcium, under these conditions, the substrate, MgATP, inhibits hydrolysis of ATP and contraction by binding to a specific relaxing site. This site is separate and distinct from the active sites where MgATP causes contraction (*4*). When calcium is added to a gel inhibited by MgATP, it triggers contraction by overcoming this substrate inhibition and allowing the contractile reactions to occur (*3, 4*).

The sensitivity of the gel to substrate inhibition and to calcium is lost after a number of different treatments: aging (*4*), partial titration of SH groups (*4, 5*), mild digestion with trypsin (*6*), special washing procedures (*7*), and heat (*8*). Certain of these treated preparations have been shown to regain their calcium sensitivity when tropomyosin is added to them (*6*). This has led to the view that the function of the relaxing site probably depends on tropomyosin in association with one of the main protein constituents—most probably with the F-actin polymer (*9*). Other less well-defined proteins have also

Table 1. Effects of dithiothreitol on heat inactivation of the relaxing site of natural actomyosin. The reaction mixture contained 0.005 mole of MgCl, 0.03 mole of KCl, and 0.06 mole of tris(hydroxymethyl)aminomethane (*p*H 7.4) per liter at 25°C. The procedure for inactivating the protein was as follows. The above solution was heated to 43°C. Actomyosin, as prepared in this laboratory (*13*), was added to a final concentration of 0.12 mg/ml. After 5 minutes, the reaction solution was cooled to 25°C and tested. To reverse inactivation, the reaction solution, heated as above, was cooled to 4°C, and DTT was added as indicated. At intervals, portions were removed and tested at 25°C. The rate of contraction was measured by recording the increase in turbidity of the actomyosin gel suspension at 545 mμ (*13*). All rates were measured at 0.002*M* ethylene glycol *bis*(aminoethylether)-*N,N'*-tetraacetic acid (EGTA) and $1 \times 10^{-4}M$ ATP in addition to the conditions described above.

Conditions	Rate of contraction (optical density change at 545 mμ per second)
Heat inactivation and protection by DTT	
Unheated protein	0
Unheated protein + 0.5 m*M* DTT	0
Unheated protein + 0.002*M* Ca^{++}	0.460
Unheated protein + 0.5 m*M* DTT + 0.002*M* Ca	.450
Heated protein	.370
Heated protein + 0.002*M* Ca^{++}	.340
Protein heated with 0.5 *mM* DTT	
Reversal by DTT	
Heated protein	0.390
Heated protein incubated with 0.5 m*M* DTT for 24 hours	0
Heated protein incubated with 5 m*M* DTT for 2 hours	0