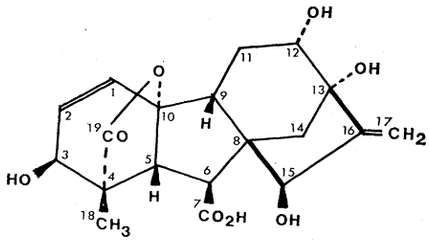
GA_{32}: A Polar Gibberellin with High Biological Potency

Abstract. *A new gibberellin, GA_{32}, purified from* Prunus *seed, has three interesting properties: (i) it is the most polar of the 34 gibberellins presently known, (ii) it is the most potent of all gibberellins tested in the barley endosperm bioassay, and (iii) it is very active in promoting the growth of unpollinated apricot ovaries with a normal ratio of length to diameter.*

The seed of two species of *Prunus* have been shown to contain a new gibberellin, GA_{32}. Yamaguchi *et al.* purified and characterized the compound from seed of peach (*P. persica* L.), and Coombe and Tate purified the same compound from seed of apricot (*P. armeniaca* L.) (*1*). The GA_{32} has the following structure:



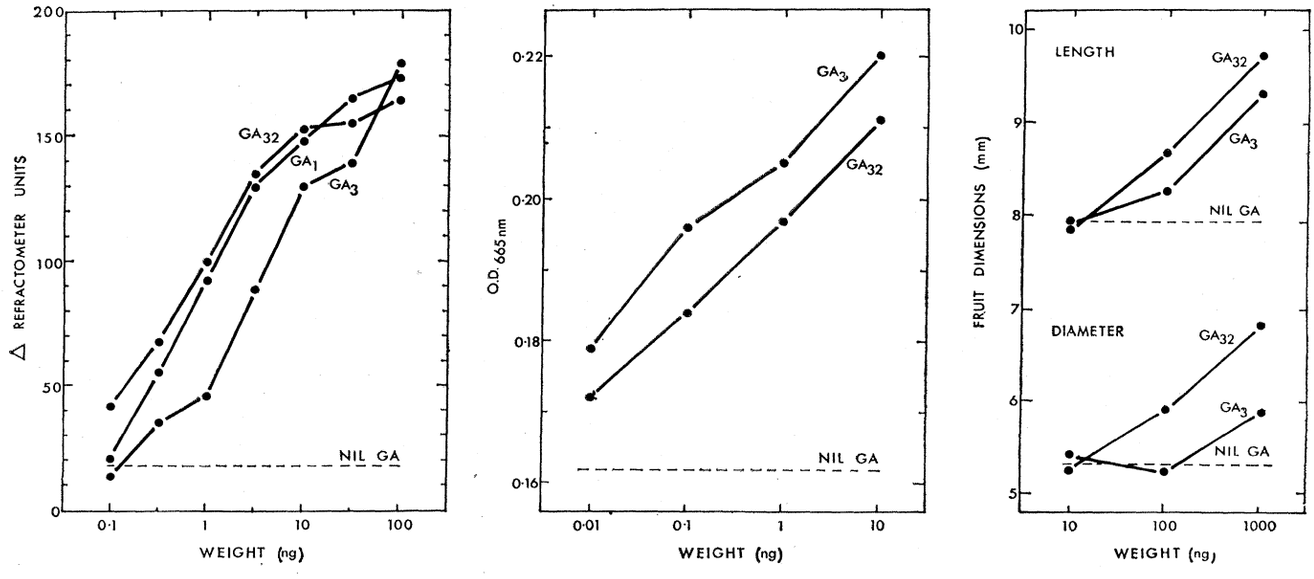
This structure is identical to GA_3 except for the hydroxyl group at carbons 12 and 15 [gibberellane numbering (*2, 3*)]. With four hydroxyl groups, it is the most polar of the free gibberellins and, chromatographically, is as polar as the glucosides of GA_3 and GA_8.

Those polar gibberellins known hitherto have been shown to possess low biological activity: free gibberellins with three hydroxyl groups, such as GA_8, have low activity (*3*), and the glucosides of GA_3 and GA_8 have been found much less potent than the corresponding free gibberellins (*4*). Evidence is presented in this report which demonstrates the high biological activity of GA_{32} in three systems—barley endosperm, *Rumex* leaf senescence, and unpollinated apricot ovaries.

The methods used for the barley endosperm bioassay were developed from those originally described (*5*). I have found a considerable saving in time, together with greater accuracy, by assessing the response as the refraction of the incubated material, rather than by measuring its reducing sugar content. The method is as follows.

Seed of barley (cultivar 'Clipper') were soaked in 50 percent (by volume) H_2SO_4 for 4 hours at room temperature, then washed 15 to 20 times in autoclaved, deionized, distilled water (sterile water), with vigorous shaking to dislodge husks. The dehusked seed were soaked in sterile water for 20 to 24 hours in the refrigerator. Endosperm pieces were prepared by cutting the seed transversely 4 mm from the end away from the embryo, as has been described (*5*). Two pieces were added to each vial. The vials, 24 by 50 mm, were dry sterilized at 160°C, and the following aqueous solutions were dispensed into them, making a total volume of 1 ml: (i) freshly prepared and autoclaved citrate-phosphate buffer to make 0.005*M* (*p*H 4.5), (ii) solution containing 25 μg each of chloramphenicol and streptomycin sulfate, and (iii) sterile water containing known amounts of gibberellin. The vials were capped and incubated 40 to 42 hours at 30°C. After incubation, the caps were removed, 9 ml of water was added, and the refraction of the diluted incubated material was measured with a Waters R4 differential refractometer (*6*) set to read digitally at maximum sensitivity, and with water in the reference cell. The change in refractometer units was obtained by subtracting the readings obtained with a reagent blank, without endosperm.

Five replicates of seven concentrations (varying in a geometric series from 0.1 to 100 ng) of GA_1, GA_3, and GA_{32} (*7*) were bioassayed. The results (Fig. 1 and Table 1) show that the response curves are parallel, that GA_{32} is 4.4 times more potent than GA_3, and that GA_{32} has significantly ($P > .05$) greater potency than GA_1. In the barley endosperm bioassay, GA_1 and GA_3 have been shown to be the most potent of nine gibberellins (*8*), and GA_1 is the most potent of 26 gibberellins in the barley amylase bioassay (*3*). The greater potency of GA_{32} compared with GA_3 has been



Figs. 1 to 3. Dose-response curves of GA_3 and GA_{32} (and in one case GA_1) in the barley endosperm bioassay (Fig. 1, left), in the *Rumex* leaf senescence bioassay (Fig. 2, center), and on unpollinated apricot ovaries (Fig. 3, right). The abscissa scales refer to concentration of GA as weight (in nanograms) per volume of test solution (1 ml in Fig. 1, 0.5 ml in Fig. 2, and 0.002 ml in Fig. 3).

found in three other similar barley endosperm bioassays; in these the relative potencies were 5.8, 2.5, and 2.4. The geometric mean of the four relative potency values is 3.5.

The *Rumex* leaf senescence test was selected because of its great sensitivity to gibberellic acid. It was performed in the manner described by Whyte and Luckwill (*9*). Four replicates of four concentrations of two gibberellins, GA_3 and GA_{32}, were used. The results (Fig. 2 and Table 1) show that, again, the response curves to the two gibberellins are parallel but that, in this case, GA_{32} is less potent than GA_3 by a factor of 5.5.

Whyte and Luckwill report that GA_3 was more active than GA_1 (8-fold), GA_7 (25-fold), and GA_4 and GA_5 (67-fold). However, it would be unwise to interpolate the activity of GA_{32} based upon bioassays done in different laboratories.

Unpollinated apricot ovaries were treated with GA_{32} to assess its significance as a hormone. On six apricot trees, cultivar 'Moorpark,' growing in the experimental orchard of the Waite Agricultural Research Institute, seven pairs of flowers were selected on separate limbs before the petals had unfolded. Neighboring flowers were removed, and, from one of each selected pair, the petals and the distal third of the style were excised. When the other marked flowers attained anthesis, their ovaries were removed and measured; fresh weights averaged 11 mg; suture diameters, 2.5 mm; nonsuture diameters, 2.1 mm; and ovary lengths, 4.2 mm. At the same time the flowers without stigmas were treated with gibberellin by excising the remainder of the style and covering the wound immediately with 2 μl of agar solution.

The solutions were prepared by dissolving GA_3 and GA_{32} in water, containing 0.2 percent (weight/volume) agar and 0.1 percent (by volume) Tween 80, to give concentrations of 10, 100, and 1000 ng of GA per 2 μl (*10*). A control without gibberellin was included. This mixture was adapted from the work of Thompson (*11*) who found it successful on strawberry flowers, while lanolin mixtures were toxic. Similarly, I found lanolin mixtures were toxic to apricot ovaries.

The diameters (mean of suture and nonsuture) and lengths of ovaries were measured 2 weeks after treatment. The results (Fig. 3 and Table 1) show that both gibberellins stimulated the growth of unpollinated apricot ovaries in amounts increasing with concentration. The response curves of length and diameter between 100 and 1000 ng are parallel. Even allowing for the uncertainty in concentration of GA_{32} (*10*), it is clear that GA_{32} is more potent than GA_3 in the induction of growth in apricot ovaries and that it causes relatively more growth in diameter. Ovaries treated with 1000 ng of GA_{32} had a ratio of length to diameter of 1.42, while those receiving a similar dose of GA_3 had a ratio of 1.59, that is, they were "slimmer." From the data of Jackson (*12*), based on measurements of fruit from the same orchard, the ratio of length to diameter of untreated fruit 14 days after anthesis was 1.45, while that of fruit from limbs perfused with GA_3 solution [15 parts per million (weight/volume), 4 days before anthesis] was 1.55. It appears, therefore, that the initial growth of unpollinated apricot ovaries induced by GA_{32} was normal with respect to overall shape.

Table 1. Bliss analyses (*5*) of bioassay data in Figs. 1, 2, and 3; S.E., standard error of the mean.

Bioassay	Slopes ± S.E.	Log potency relative to GA_3 ± S.E.
Barley endosperm (Fig. 1, excluding highest two concentrations)		
GA_3	57.3 ± 5.0	0
GA_1	65.4 ± 4.3	0.44 ± 0.08
GA_{32}	57.7 ± 3.6	0.64 ± 0.08
Rumex *leaves (Fig. 2, all values)*		
GA_3	0.0133 ± 0.0023	0
GA_{32}	0.0132 ± 0.0031	1.25 ± 0.35
Apricot ovaries (Fig. 3, excluding 10 ng of GA_3 values)		
Length		
GA_3	1.05 ± 0.64	0
GA_{32}	0.93 ± 0.26	0.46 ± 0.38
Diameter		
GA_3	0.69 ± 0.22	0
GA_{32}	0.78 ± 0.12	1.03 ± 0.21

At the time the above measurements were made, fruits treated with GA_{32} were about 30 percent smaller in length and diameter than normal fertilized fruits. Within a further 2 weeks these fruits abscissed.

It is clear that GA_{32} is exceptional in being the most polar of the known nonconjugated gibberellins and at the same time being highly active biologically. In the barley endosperm bioassay it is the most potent gibberellin tested. Obviously, the two extra hydroxyl groups at carbons 12 and 15, which confer the increased polarity, do not interfere with the active sites of this molecule. The fact that the substituents and bonding in ring A are identical to those in gibberellic acid (GA_3) strengthens the suggestion made by Crozier *et al.* (*3*) that high activity in gibberellin molecules is associated with a lactone bridge between C-4 and C-10 and a hydroxyl group attached to C-3 but not to C-2, or alternatively a double bond or epoxide between C-2 and C-3.

In extracts of apricot seed and pericarp, GA_{32} accounts for most of the gibberellin-like activity, and the amount of activity is correlated with the rate of cell expansion in these tissues (*13*). Moreover, GA_{32} applied exogenously is highly potent in stimulating the growth of unpollinated apricot ovaries, and the growth is normal in shape. That the growth was less than in fertilized fruits is probably due to the fact that GA_{32} was applied only once. If GA_{32} is the pericarp cell-enlarging hormone, the present data suggest that the seed is its source.

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References and Notes

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6. Supplied by Waters Associates Ltd., Portwood, Stockport, Cheshire, England; also available from Waters Associates Ltd., Framingham, Mass.
7. GA_1 kindly supplied by B. E. Cross; GA_3, by Merck Ltd., Rahway, N.J.; GA_{32} purified from apricot seed by Coombe and Tate [see (*1*)] as the ammonium salt and converted to the acid using H^+ exchange resin.
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10. The amounts of GA_{32} used in this bioassay were measured, not by direct weighing, but by the determination of biological activity compared with that of GA_3 in a barley endosperm bioassay with the relative potency value of 3.5. They should therefore be regarded as having a variation of $\times 2$ to 1/2.
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