

Ten Foo Wei – Dec 23rd 2013

Gibberellic acid (GA)

Discovery and Structure

Cautionary Tale

- ◆ *Baka-nae* = foolish seedlings in Japanese (馬鹿苗)
- ◆ Characteristics:
 - ◆ Visibly etiolated
 - ◆ Chlorotic
- ◆ Infertile with empty panicles = no edible grains
- ◆ Topple over = unharvestable or death



http://agrin.jp/hp/inasaku/ikubyou_byouga.htm



<http://www.rkmp.co.in/content/symptoms-of-foot-rot-bakanae-disease-in-karnataka-0>

Since time immemorial, before *Momo-taroh* defeated the *oni* (demon) and even before *Genji* went on his adventure, farmers in Asia were well aware of a certain disease that infects rice plants.

These infected plants would grow excessively tall, dwarfing their siblings and towering over the rest of the rice in the paddy field.

In their fast and furious race to reach the heavens however, eventually they succumb to their own weight, fell over, and become unharvestable.

Eventually, the name calling started, and soon the Japanese farmers started to call these ambitious seedlings *baka-nae* (foolish seedlings), *aho-nae* (stupid seedlings), *kuso-nae* (shit seedlings), and etc.

Compared to their normal, smarter siblings, these foolish seedlings are visibly etiolated, which means that they are taller and more slender. They are also chlorotic, appearing more yellowish compared to the green colored normal seedlings.

At best, they are infertile with empty panicles, producing no edible grains;

At worst, they are incapable of supporting their own weight and will eventually topple over and die.

Cautionary Tale



http://www2.pref.iwate.jp/~hp2088/park/kikaku/48th%20images/48th_makimono01.html

These foolish seedlings, despite their funny names, are no funny matters.

In fact, *baka-nae* is a force to be reckoned with, as the fungus affects rice crops in Asia, Africa and even North America.

In epidemic cases yield losses may reach as high as 20% or more.

In other words, these foolish seedlings are threatening the lives of many people around the world, especially those who eat rice as staple food.

Brief history

- ◆ 1898 – Shotaro Hori: First scientific paper **published**
 - ◆ *Fusarium heterosporum*
- ◆ 1912 – **Kenkichi Sawada**: “The Diseases of Crops in Taiwan”
 - ◆ Elongation due to **stimulus derived from fungal hyphae**
- ◆ 1926 – **Eiichi Kurosawa**: **Confirmed** evidence that a **chemical substance** was involved
- ◆ 1931 & 1935 – Wollenweber and Reinking:
 - ◆ *Gibberella fujikuroi*



Professor Shotaro Hori

http://search.ssccommons.org/object/557729463_7729463_1180193

In order to save the rice eaters, many brave scientists embarked on an epic quest to purge these foolish seedlings from the land, and after countless attempts, the first scientific paper on the cause of *baka-nae* was finally published in 1898 by Shotaro Hori.

Furthermore, he demonstrated that the symptoms were induced by infection with a fungus belonging to the genus *Fusarium* by inoculating the isolated fungus onto healthy rice seedlings. Unfortunately, the taxonomic position of the *baka-nae* fungus had been a topic of long discussions. Nevertheless, he postulated that it might probably be *Fusarium heterosporum*.

It wasn't until 1912 that another guy called Kenkichi Sawada published a paper titled “The Diseases of Crops in Taiwan” in which he suggested that the elongation in rice seedlings infected with *baka-nae* fungus might be due to a stimulus derived from fungal hyphae.

Subsequently, in 1926, another guy called Eiichi Kurosawa found that culture filtrates from dried rice seedlings caused marked elongation in rice and other sub-tropical grasses. He concluded that *baka-nae* fungus secretes a chemical that stimulates shoot elongation but inhibits chlorophyll formation and suppresses root growth.

Later, the taxonomic position of the *baka-nae* fungus was finally clarified in the papers of Wollenweber (1931) and Reinking (1935), where the imperfect stage of the fungus responsible for the *baka-nae* disease was named *Fusarium moniliforme* and the perfect stage was named *Gibberella fujikuroi*. The latter name has been widely used ever since.

Eiichi Kurosawa

1. Cultured Bakanae fungus on solid medium consisting of powdered dried rice seeds
2. Cultured media was extracted with boiling water
3. Cultured fungus in media supplemented with the water extract of rice straw
4. Fungal cultures were passed through a sterile filter to remove Bakanae fungus → fungus free solution
5. Rice seedlings grown by water culture using media containing extracts of culture medium or the fungal free solution obtained from liquid culture
6. PROFIT!!! → confirmed these samples induce elongation of leaf sheath of rice seedlings

So this is basically what Kurosawa did back in the good old days.

Eiichi Kurosawa

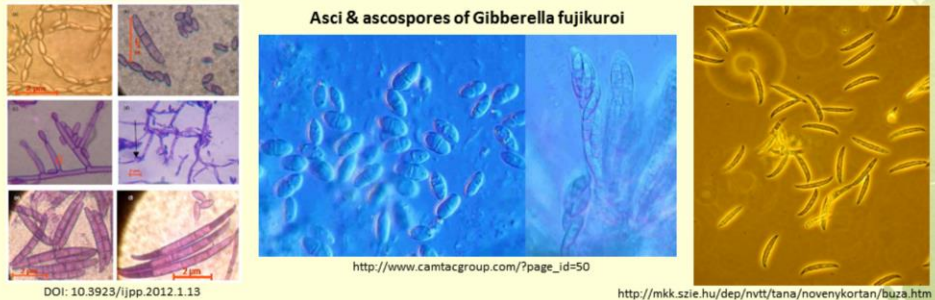
1. Bakanae fungus secretes the toxin which induces elongation of rice
2. The toxin not only stimulates the growth of the leaf sheath, but also inhibits the formation of chlorophyll and roots
3. Toxin induces similar effects on other plants
4. The toxin changes little after being kept at 100°C for 4 hours
5. Specific feature of Bakanae fungus → differentiate it from other similar fungi
6. Resistance of rice to the toxin is variable → dependent on cultivar
7. Rice does NOT produce the anti-toxin factor

This was a major breakthrough, as after the paper was published, the conclusions drawn from these experiments were confirmed by other plant pathologists.

Further reports on culture conditions to produce the toxin and its chemical nature were also published.

Teijiro Yabuta

- ◆ 1934 – isolated a crystal of a substance which inhibits the growth of rice from the cultured filtrate of the bakanae fungus
 - ◆ **Fusaric acid** → 5-n-butylpicolinic acid
- ◆ 1935 – proposed the name “**gibberellin**”
- ◆ 1938 – Yabuta and Sumiki: Isolation of gibberellin in crystalline form (pale yellow) → gibberellin A, “B”



Kurosawa had a buddy named Teijiro Yabuta, who received a lot of useful information and advice on the fermentation of the *baka-nae* fungus.

As it was becoming hip to be doing researches about *baka-nae* and all the cool guys were doing it, his group also started isolation studies on the toxin.

And then.. The fateful day dawned one fine day in 1934.

Yabuta was the first person to isolate a crystal of a substance which inhibits the growth of rice seedlings at all concentrations tested from the cultured filtrate of the *baka-nae* fungus.

They named this awesome crystal.... FUSARIC ACID! (its structure was later found to be 5-n-butylpicolinic acid)

The formation of fusaric acid in culture filtrates was suppressed by changing the composition of culture medium. As a result, a non-crystalline solid was obtained from the culture filtrate that stimulated the growth of rice seedlings. He proposed the name “gibberellin” for this compound in 1935. Then, in 1938, Yabuta and his associate Yusuke Sumiki finally succeeded in crystallizing a pale yellow solid to yield gibberellin A and gibberellin B.

It wasn't until many years later that they found out that their samples were contaminated, and that gibberellin A was actually the inactive form of gibberellin B.

Realizing their silly mistake, they changed the “B” in gibberellin B's name to A and pretended that gibberellin B never existed in the first place.

Gibberellic acid (I)

- ◆ In the US research on gibberellins began after WW2
 - ◆ Using bean seedlings
- ◆ 1951 – Sumiki visited the US
 - ◆ Isolated gibberellin-x
- ◆ In UK – researchers isolated a new gibberellin
 - ◆ → gibberellic acid
 - ◆ Physical properties different from Japanese gibberellin A
- ◆ Gibberellic acid = Gibberellin-x !!

Across the Pacific, the first research on gibberellins began in the United States after WW2 (using bean seedlings). They tried to carry out large scale fermentations to produce pure gibberellin for agricultural uses but unfortunately, the initial fermentations were inactive.

Then, in 1951, Sumiki visited the US (to watch the first NBA All-Star game in Boston Garden maybe?) and made some new friends.

After returning to Japan, he sent some new cultures to the US but these were proved inactive too. Eventually, the problem was traced to the lack of magnesium in the culture medium they used. Finally, good yields of gibberellin were obtained when the culture medium was supplemented with magnesium sulphate.

They were surprised when the physical properties of gibberellin isolated from these fermentations were found to be different from those reported by the Japanese, and they named this new compound gibberellin-x. Around the same time, another group of researchers in the UK also isolated a new gibberellin which was given the name gibberellic acid.

The UK and US teams exchanged their samples and they found that both gibberellin-x and gibberellic acid have identical chemical and physical properties.

And that's when they realized that they found the same thing, that gibberellin-x was actually gibberellic acid!

Gibberellic acid (III)

- ◆ 1955 – Members of Sumiki group
 - ◆ Separated methyl ester of gibberellin A
 - ◆ → gibberellins A1, A2, A3
- ◆ Surprise! Gibberellin A3 = Gibberellic acid
- ◆ 1957 – Takahashi
 - ◆ Isolated gibberellin A4
- ◆ 1950s
 - ◆ UK team demonstrated presence of gibberellin-like substances in higher plants
 - ◆ US team reported gibberellin-like substances in maize
 - ◆ Isolation of crystalline gibberellin A1, A5, A6, A8 from runner bean, *Phaseolus coccineus* (formerly *Phaseolus multiflorus*)

Fast forward to 1955, members of Sumiki group finally succeeded in separating the methyl ester of gibberellin A into 3 components.

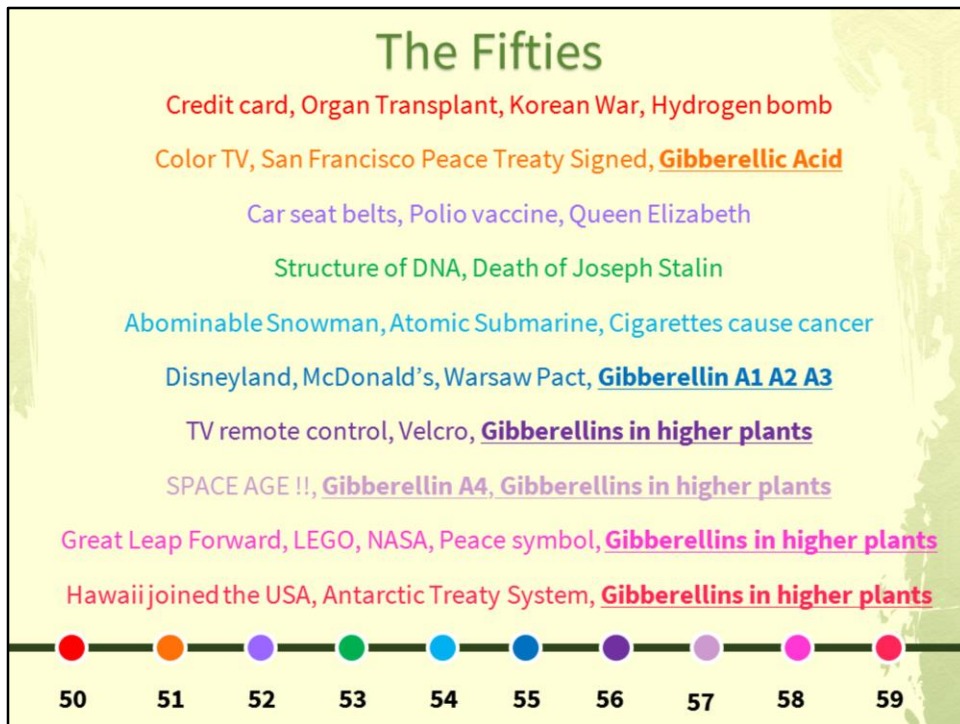
The corresponding free acids were obtained and named gibberellins A1, A2 and A3.

And then.. They got another surprise!

It turned out that gibberellin A3 was identical to the gibberellic acid isolated by the UK team few years back.

Later, in 1957, Takahashi (a member of Sumiki group) isolated gibberellin A4.

Soon, both teams reported the presence of gibberellin-like substances in higher plants like maize, runner bean and etc.



The Fifties was a decade dominated by clashes between communism and capitalism.

1950 marked the beginning of the Korean War and the US commissioned the first Hydrogen Bomb.

1951 - Color TV was introduced; San Francisco Peace Treaty was signed, formally ending Japan's position as an imperial power; **Gibberellic acid was discovered in the same year by the UK team.**

1952 - The Polio vaccine was invented, and Princess Elizabeth became the Queen.

1953 - Watson and Crick discovered the molecular structure of DNA, and Joseph Stalin died.

1954 - The British Daily Mail commissioned an expedition to retrieve the abominable snowman. The same year, scientists reported that cigarettes actually causes cancer.

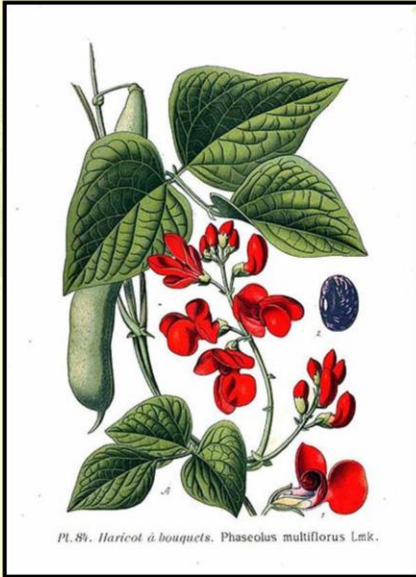
1955 - Disneyland opened; McDonald's was franchised; The Warsaw Pact was signed; **Gibberellins A1 A2 A3 were identified by Sumiki's group.**

The Vietnam War started in 1956. The next year, in 1957, the launch of Sputnik by the Soviets marked the beginning of the Space Age.

In 1958, Mao Zedong initiated the not-so-Great Leap Forward; LEGO was introduced; And the peace symbol was created; Then, in the final year of the Fifties; Barbie was launched; Hawaii became the 50th state of the Union; The Antarctic Treaty System was also established in the same year; The beginning of decolonization in Africa and Asia (including Malaysia, as well as the end of the occupation of Japan) also occurred in this decade, and accelerated in the 60s.

During this exciting period as the world was progressing into a new era, chemists and plant researchers, not wanting to lose to their physics/astronomy/humanities/etc rivals, published reports of evidences that gibberellins were naturally occurring substances in higher plants. (Interestingly.. If you look at the timeline.. Those reports were published right after Disneyland opened and McDonald's started springing up everywhere...)

Runner beans



Pl. 89. Haricot à bouquets. Phaseolus multiflorus Lmk.

Atlas des plantes de France. 1891



Runner bean & prosciutto pasta

- 175g tagliatelle
- 200g runner beans, trimmed and cut into short lengths
- 3 slices prosciutto
- 1 tbsp olive oil
- 2 rounded tbsp half-fat crème fraîche

<http://www.bbcgoodfood.com/recipes/4718/runner-bean-and-prosciutto-pasta>

So here's a nice illustration of runner beans and..

Here's a recipe for those who wanna make a delicious meal with runner beans..



The Fifties came to an end and now we're entering The Sixties. The 1960s was a period of radical changes. If the 50s was an amazing decade, then I would say the 60s was a FABULOUS decade.

The whole decade was plagued by wars, conflicts, disasters and assassinations. The Cold War, Cultural Revolution in China, assassination of JFK and Martin Luther King Jr a few years after his "I Have A Dream" speech and etc. just to name a few. In fact, the Cuban Missile Crisis in 1962 nearly erupted into a full scale global nuclear conflict that will most likely end in the mutual destruction of the world.

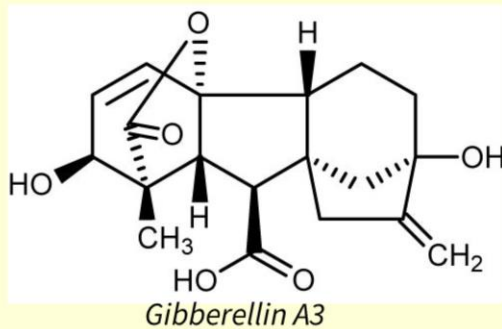
However, it was also this turbulent decade that sparked a social revolution with the rise of many movements and revolutions including anti-war, civil rights, feminism, gay rights movements. This era also marked the end of colonialism by the great European empires that once dominated seas and continents, with 32 countries gaining independence from their European overlords. The rise of counterculture movement among the youth also gave rise to the hippie movement and the British Invasion of music, the notable ones including The Beatles, The Rolling Stones and Pink Floyd. It was also during this decade that the bikini, mini-skirt and afro became the rage.

Of course, huge progresses were also made in science and technology, especially in the fields on electronics.

Oh and, mankind set foot on the moon before this decade came to an end too, though they didn't find any bunnies up there making *mochi*.

Gibberellic acid (III)

- ◆ 1960s
- ◆ 1968 – J. MacMillan and Takahashi
 - ◆ All gibberellins should be assigned numbers as **gibberellin A1-x**
 - ◆ Irrespective of origin
- ◆ No. of gibberellins identified
 - ◆ **126!! (as of 2003)**



Anyway, let's go back to the gibberellins now.

The 1960s also marked the gibberellin boom, where the number of gibberellins isolated from fungal and plant origins rapidly increased.

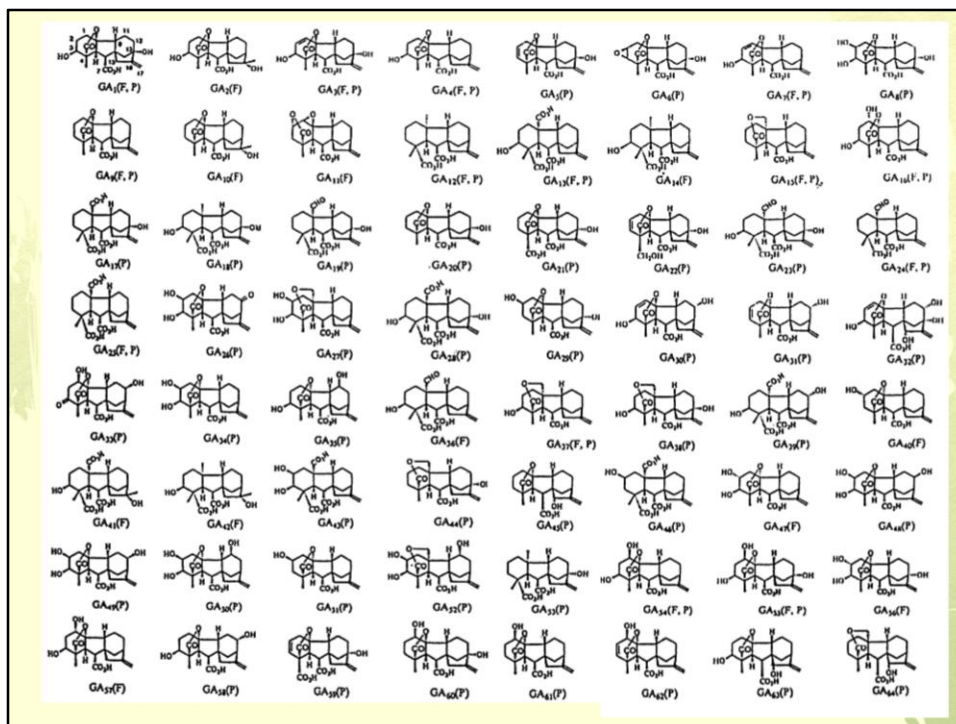
Soon, the scientific world was engulfed in the “gibberellic acid fever” and waves after waves of researchers went on a gibberellic acid discovery and naming spree.

Many new species of gibberellic acid appeared, including bamboo gibberellin, banana gibberellin, cucumber gibberellin, *hourensou* gibberellin and etc.

Finally, in 1968, J. MacMillan and Takahashi decided they had enough and reached an agreement that all gibberellins should be assigned numbers as gibberellin A1 to AX irrespective of their origins.

And that's it. No more funny names for the gibberellins too.

Over the years, many more gibberellins have been identified and we have identified at least 126 different gibberellins now.



Here you can see all the different kinds of gibberellins.

As you can see, they come in many different shapes and sizes.



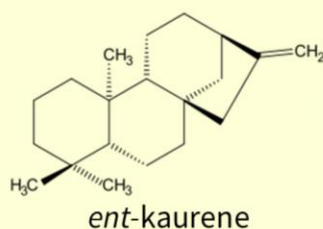
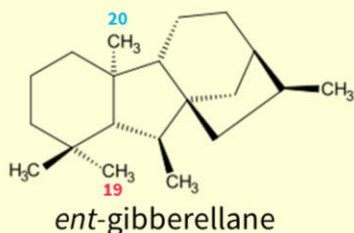
There are sooooo many of them and I can't even fit all of them in one slide.

And if you want to be very best like no one ever was..

Well, its time to start training as we gotta catch em all!

Structure

- ◆ All known gibberellins are **diterpenoid acids**
 - ◆ Formed from 4 isoprenoid units
 - ◆ All have 19 or 20 C
 - ◆ Grouped into 4 or 5 rings systems
- ◆ **Synthesized in the terpenoid pathway in plastids**
- ◆ **Modified in endoplasmic reticulum and cytosol to reach biologically-active form**
- ◆ **Derived via the *ent*-gibberellane skeleton**
- ◆ **Synthesized via *ent*-kaurene**
- ◆ Named GA₁ ... GA_n in order of discovery



Finally, we're done with the history of gibberellins and now we will move on to its structure.

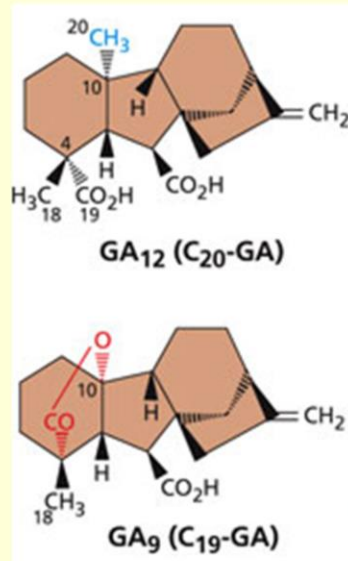
Unlike the classification of auxins which are classified on the basis of function, gibberellins are classified on the basis of structure as well as function.

All gibberellins are diterpenoid acids derived from the *ent*-gibberellane skeleton.

They all have either 19 or 20 carbon units grouped into either four or five ring systems.

Structural modifications

- ◆ $20C \rightarrow C_{20}$ -GAs (eg. GA₁₂)
- ◆ $19C \rightarrow C_{19}$ -GAs (eg. GA₉)
 - ◆ C-20 lost by metabolism
 - ◆ In nearly all C_{19} -GAs, carboxyl at C-4 forms lactone at C-10



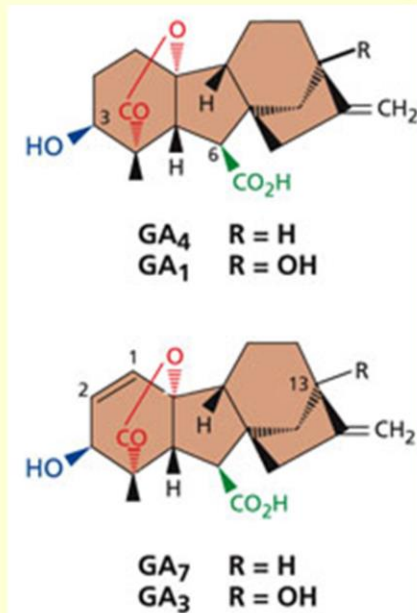
Gibberellins with 20 carbons are called C₂₀-GAs while those with 19 carbons are called C₁₉-GAs.

Pretty straightforward eh?

In C₁₉-GAs, the carbon 20 was lost by metabolism, and in nearly all of them, the carboxyl at carbon 4 forms a lactone with carbon 10

Structural modifications

- ◆ Insertion of additional features
 - ◆ -OH groups, double bonds
- ◆ Bioactive GAs = GA1, GA3, GA4, GA7
 - ◆ All C19 GAs
 - ◆ 4,10 lactone
 - ◆ Carboxylic acid @ C6
 - ◆ -OH group @ C3 in β -orientation



Other structural modifications include the insertion of additional features such as hydroxyl group or double bonds.

The position and stereochemistry of these functional groups can have profound effects on biological activity of the gibberellin.

The GAs with the highest intrinsic biological activity were among the first to be discovered, and they are all C19-GAs.

They all possess 4,10-lactone, a carboxylic acid at C6 and a hydroxyl group at C3 in β -orientation.

Structural modifications

- ◆ GA receptor interacts with these functional groups in a bioactive GA molecule
- ◆ Hold GA in receptor pocket, allowing a lid to close in place
 - ◆ Facilitates subsequent interactions of the GA receptor with other proteins
- ◆ Other features:
 - ◆ Double bond between C1 and C2 (GA7, GA3)
 - ◆ -OH group at C13 (GA1, GA3)
 - ◆ Neither help nor hinder GA binding in receptor pocket
- ◆ Presence of 2 β -OH group
 - ◆ Prevents hydrophobic surface of receptor lid from closing, and thus renders a GA inactive

The amino acid residues in the GA receptor interact with these functional groups in a bioactive GA molecule by holding and tucking the GA into its pocket.

This causes a conformational change and allows a lid to close in place.

This allosteric modification of the GA receptor upon binding an active GA also facilitates subsequent interactions of the GA receptor with other proteins.

Although bioactive GAs may possess other features such as hydroxyl groups at C13 and/or double bond between C1 and C2, these groups neither help nor hinder the GA binding in the receptor pocket.

On the other hand, the presence of 2 β -OH group prevents hydrophobic surface of the receptor lid from closing and thus, renders the GA inactive.

Function

- ◆ Active gibberellins → physiological effects
 - ◆ Depends on type and species of plants
- ◆ Stimulate stem elongation by **stimulating cell division** and **elongation**.
- ◆ **Stimulates bolting/flowering** in response to long days.
- ◆ **Breaks seed dormancy** in some plants which require stratification or light to induce germination.
- ◆ **Stimulates enzyme production** (α -amylase) in germinating cereal grains for mobilization of seed reserves.
- ◆ **Induces maleness** in dioecious flowers (sex expression).
- ◆ Can **cause parthenocarpic** (seedless) fruit development.
- ◆ Can **delay senescence** in leaves and citrus fruits.

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