VITAMINS

From Theory to Industrial Synthesis in 100 Years

Nutrition Facts

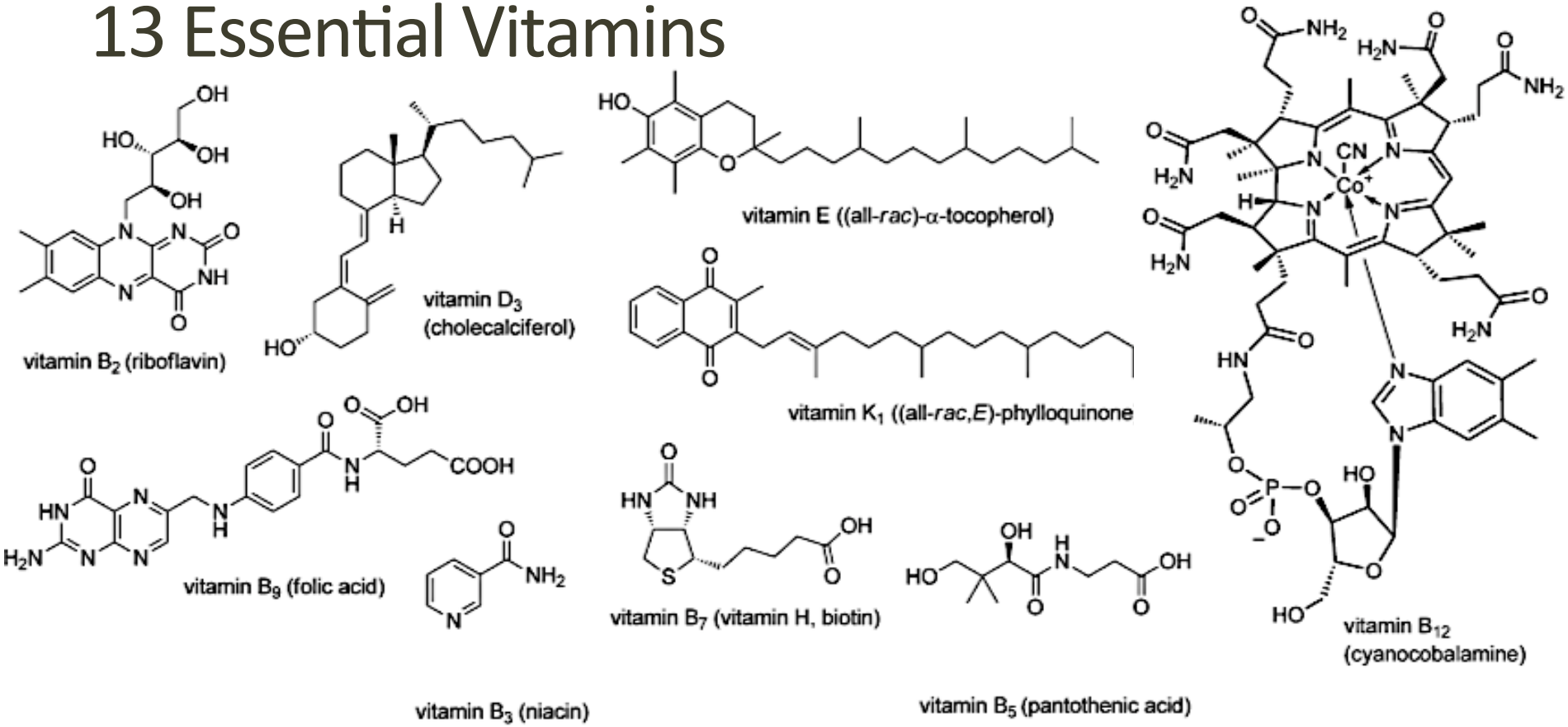
Serving Size 1 Banana (130g)
Servings per Container 1

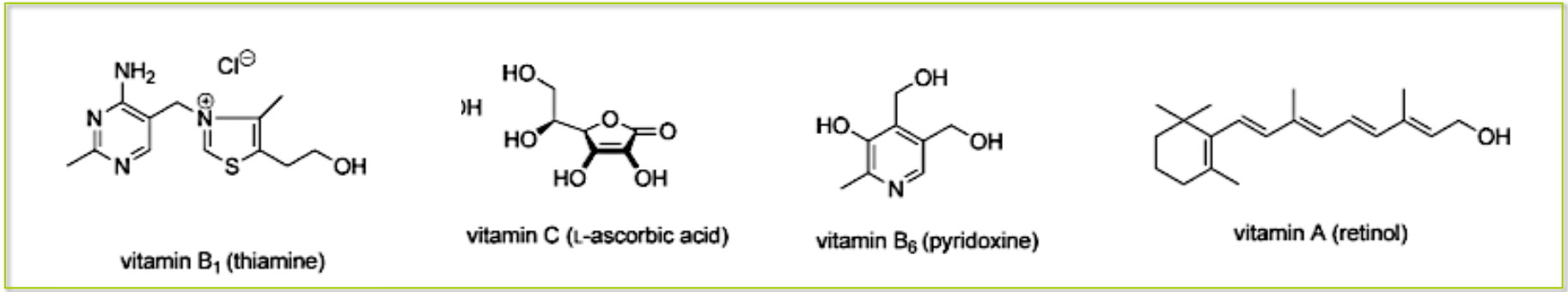
Amount Per Serving	
Calories 300	Calories from Fat 130
	% Daily Value*
Total Fat 16g	**24%**
Saturated Fat 8g	**39%**
Trans Fat 0g	
Cholesterol 0g	**0%**
Sodium 15mg	**1%**
Potassium 320mg	**9%**
Total Carbohydrate 44g	**15%**
Dietary Fiber 4g	**16%**
Sugars 32g	
Protein 2g	
Vitamin A	2%
Vitamin C	15%
Calcium	2%
Iron	6%
Riboflavin (Vitamin B2)	4%
Vitamin B6	15%
Folate	4%
Magnesium	6%
Copper	4%
Manganese	10%

* Percent Daily Values are based on a 2000 calorie diet. Your daily values may be higher or lower, depending on your calorie needs:

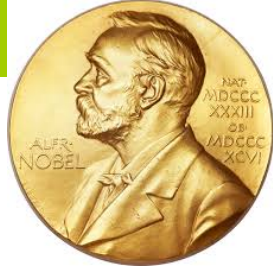
	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

13 Essential Vitamins





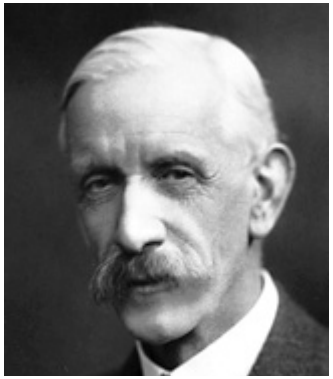
Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.



No Simple Task But High Rewards

1929 – Discovery of Vitamins

Christiaan Eijkman



Sir Frederick Gowland Hopkins

1934 – Liver Therapy of Anaemias (B_{12})

George Hoyt Whipple

George Richards Minot

William Parry Murphy

1937 – Vitamins A, B_2, and C

Paul Karrer

Walter Norman Haworth

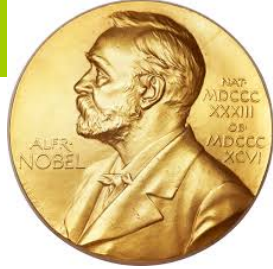
Albert von Szent-Györgyi Nagyrápolt

1938 – Defining B Complex

Richard Kuhn

Souganidis, E. *Ann. Nutr. Metab.* **2012**, *61*, 265.; Photos from www.nobelprize.org

No Simple Task But High Rewards



1943 – Vitamin K

Henrik Carl Peter Dam

Edward Adelbert Doisy

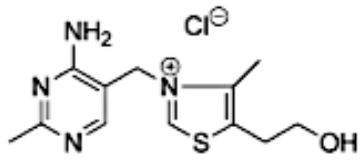
1957 & 1964 – Structure of B_{12}

Lord (Alexander R.) Todd

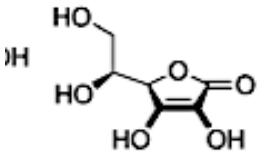
Dorothy Crowfoot Hodgkin

Souganidis, E. *Ann. Nutr. Metab.* **2012**, *61*, 265.; Photos from www.nobelprize.org

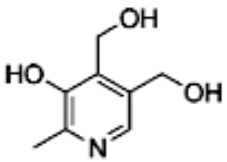
A Multidisciplinary Effort



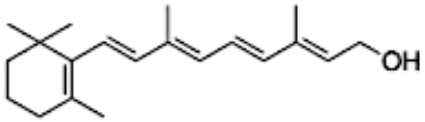
vitamin B_1 (thiamine)



vitamin C (L-ascorbic acid)



vitamin B_6 (pyridoxine)

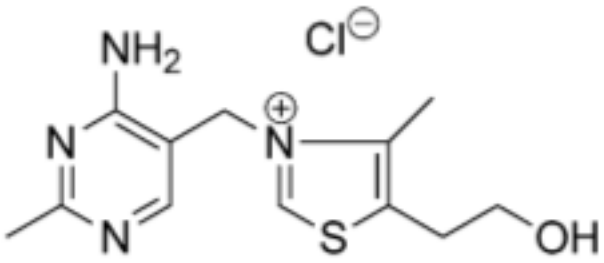


vitamin A (retinol)

- The discovery of vitamins, their structural elucidation, and ultimate application in everyday life, is a result of a wide variety of scientific study from all over the globe
- Organic chemical synthesis has been vital to our ability to prevent deficiency diseases and promote healthy growth through fortified foods and supplements
- World-wide demand for large quantities of vitamins has driven huge advances in their large scale preparations, both through fermentation (biotechnology) and chemical synthesis

Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

Thiamin (Vitamin B_1)



vitamin B_1 (thiamine)

Structure

- Bioactive form is pyrophosphate
- Supplied as the stable HCl salt
- Oxygen and UV sensitive
- Water soluble (loss in cooking)

Sources

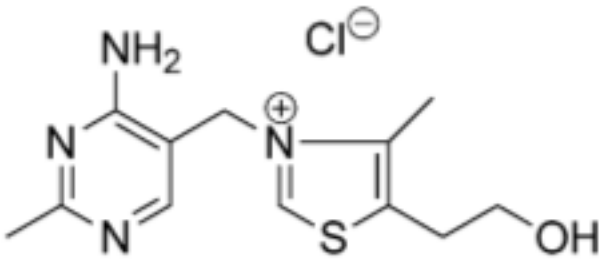
- Animal = organ meat, lean pork
- Plants = unrefined grain, nuts, legumes
- Not abundant in chicken, fruit, or milk
- Fortified into flour, bread, corn

Storage in Body

- Daily Recommended Intake 1.4 mg
- Requirement relative to energy consumption of individual
- Total body pool ~30 mg
- $t_{1/2}$ ~ 1h
- Localized in heart, liver, kidney, and brain

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Thiamin Biology



vitamin B_1 (thiamine)

Key Roles

- Coenzyme in 24+ different enzymes (decarboxylases & transketolases)
- Nature's NHC catalyst
- Component of neural membranes

Deficiency

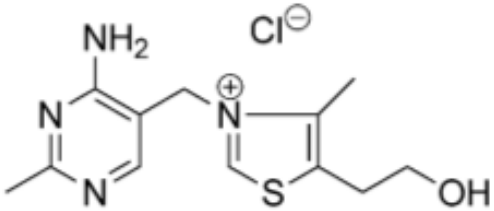
- Generally coincident w/ other B vitamin deficiencies
- Common now in developing world where refined grain/rice is a dietary staple
- Common in alcoholics (industrialized world)
- Children who eat only snack food

Initial Signs of Deficiency

- Exhaustion, emotional instability, irrational behavior, depression
- Loss of weight
- Decreased elasticity of intestinal wall (constipation, loss of appetite)

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Beriberi Plagues Southeast Asia



vitamin B_1 (thiamine)

1800s

- Descriptions of the disease date back to 10th century China, but reemerged with the industrial processing of rice
- Recognized as not contagious, and did not show a fever/loss of appetite like infections

Progression of disease:

"wet beriberi"

edema ("dropsy")
heart failure

Weak legs
(loss of feeling & strength)

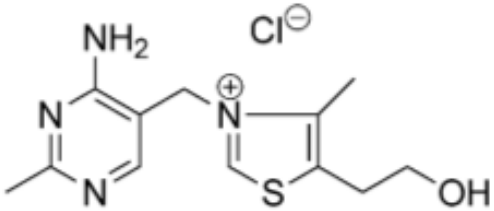
"dry beriberi"

emaciation
inability to hold up feet/hands
loss of voice

Afflicted isolated populations with restricted diets (army, students) and Asian cultures w/ polished rice

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 219.

Search for Cause of Beriberi



vitamin B_1 (thiamine)

1803: Robert Christie (Inspector General of Sri Lanka Hospitals)

States cause as nutritional deficiency (like scurvy) although not effectively treated with citrus but rather animal foods

1868: French Naval Surgeon Review

Also concluded beriberi was a result of malnutrition

1880s: Cornelius Pekelharing & Cornelius Winkler

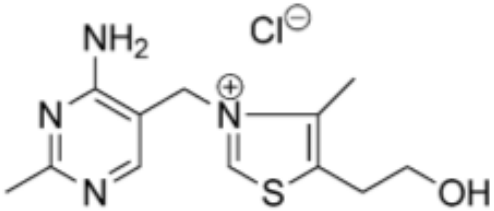
Pathologists dispatched by Dutch government to East Indies to cure outbreak in beriberi within troops

↓

"Beriberi has been attributed to an insufficient nourishment…but the destruction of peripheral nervous system on such as large scale is not caused by hunger…The true cause must be coming from outside."

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 219.

Christiaan Eijkman



vitamin B_1 (thiamine)

Remained in Dutch East Indies to continue to find infectious agent

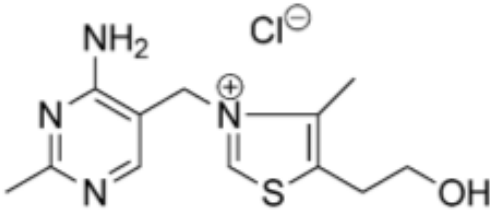
Studied beriberi occurrence in chicken populations

- Observed that uninfected chickens (controls) still developed "polyneuritis"
- Widely separated populations and ruled out cross-infection
- Shortly thereafter, all of his chickens started getting better…. (?)
- Instead of giving up, Eijkman identified that the person feeding his chickens had changed the food source from leftover white rice to brown rice
- When repeated, white rice resulted in disease, while brown rice diet did not

Despite this evidence, Eijkman did not believe it was caused by a deficiency, rather interpreted this as 'autointoxication'

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 219.

Gerrit Grijns



vitamin B_1 (thiamine)

Eijkman's replacement in Dutch East Indies

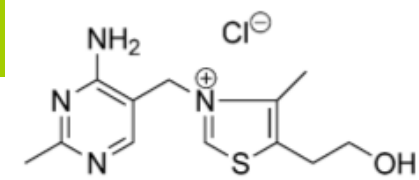
- Resumed Eijkman's work, focusing on isolating essential component from rice polishings
- Never successful in isolation, concluded that processing must destroy nutrient
- Identified that other foods (mung beans) could prevent beriberi in the chicken population (later confirmed in prison populations)

"There occur in various natural foods, substances which cannot be absent without serious injury to the peripheral nervous system… These substances are easily disintegrated which…shows that they are complex substances and cannot be replaced by simple chemical compounds"

Credited with proper interpretation of Eijkman's work

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 219.

Casmir Funk's "Vital Amine"



vitamin B_1 (thiamine)

1911: Cashmir Funk (Polish chemist)

Rice-Polishings (54 Kg) $\xrightarrow[\text{HCl (2-5\%) in alcohol}]{\text{extraction}}$ "fat-like" substrance (347 g) $\xrightarrow[\text{then extract w/ ether}]{\text{decant}}$ aqueous solution (~ 17 L) $\xrightarrow{\text{sucessive precipitations}}$

test on pigeons with polyneuritis

Alcoholic solution of extract treated with mercuric chloride = alcoholic filtrate (3)

(then Hg removed by H_2S)

ppt. of crystals which on recrystallising from H_2O gave = H_2O filtrate (2); crystalline substance (1)

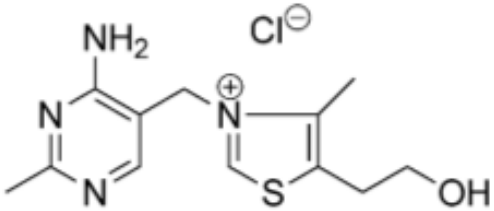
All three cure pigeons(!!!)

- Essential substance not more than 1 g / Kg of rice
- Isolated substance w/ 4 mg of nitrogen will cure pigeons
- Precipitated compound by phosphotungstic acid & $AgNO_3$ by elemental analysis propose formula of $C_{17}H_{18}O_4N$

Because it contained an amine, and did not match any known amino acids, he named it a "vital amine"

Funk, C. *J. Physiol.* **1911**, *43*, 395.

Thiamin Structure Elucidation



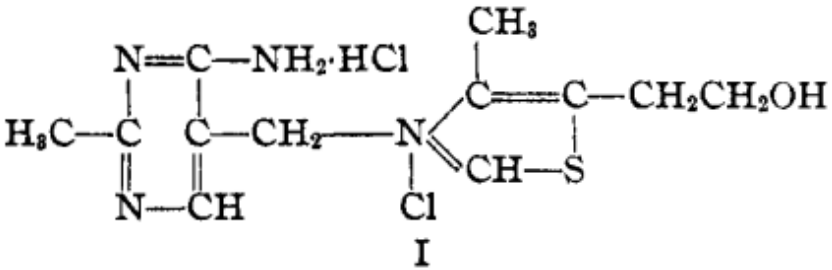
vitamin B_1 (thiamine)

1932: Adolf Windaus (German Scientist)

First person to recognize that the compound contained sulfur, and correctly proposed $C_{12}H_{18}N_4OSCl_2$ as the molecular formula

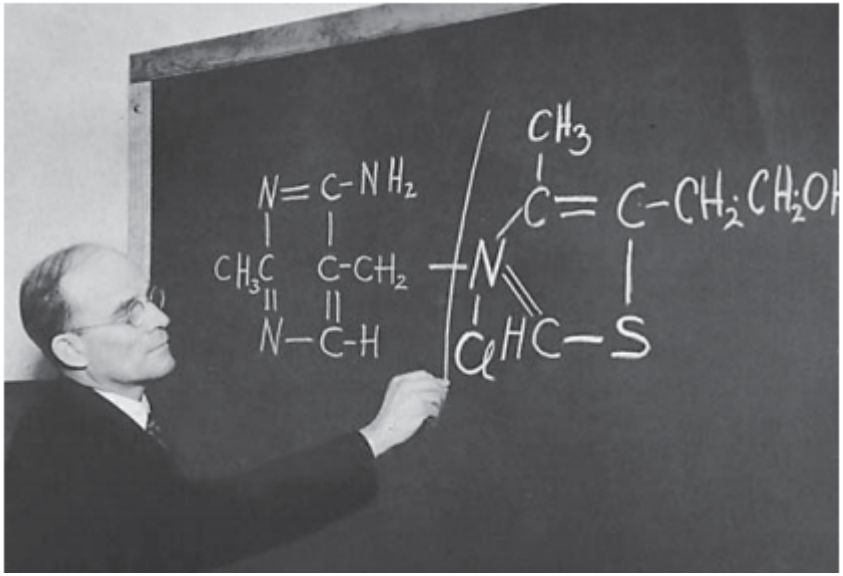
1934: Robert R. Williams (German Scientist)

First to propose correct molecular structure



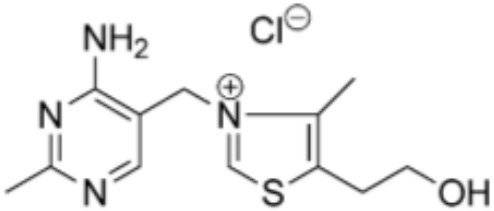
Fortuitous discovery that treatment with sodium sulfite quantitative split Vitamin B_1 into 2 components (pyrimidine II & thiazol side chain III):

Started this work on this sabbatical days during the Great Depression, working in a hospital basement and raise pigeons in his garage

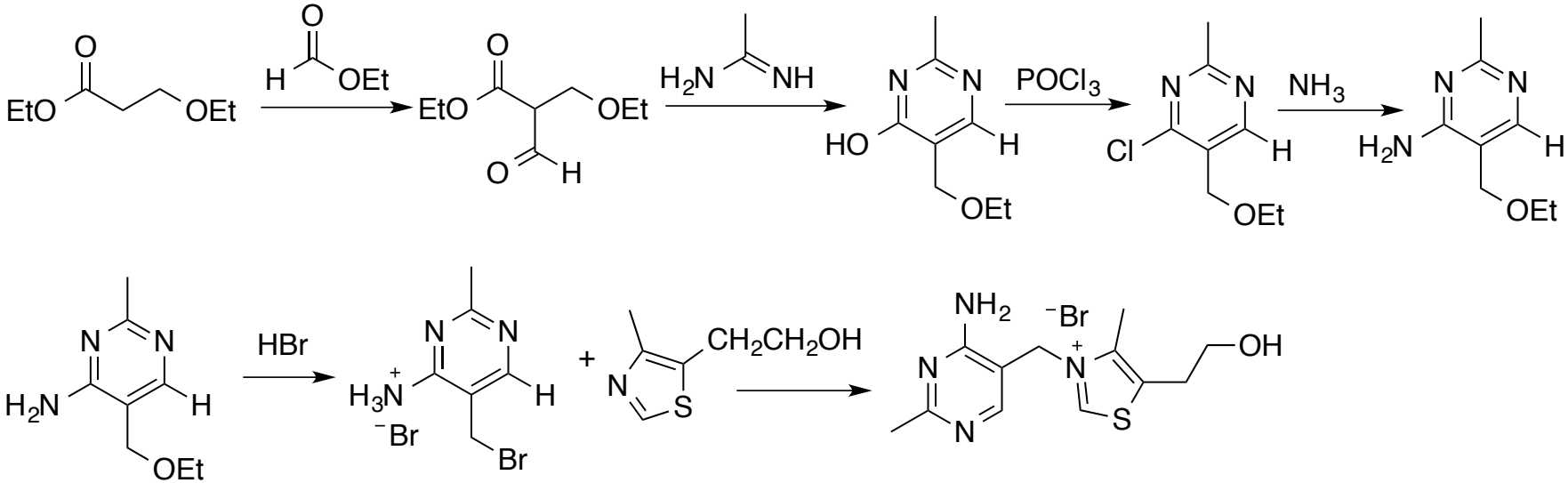


Williams, R. R. *J. Am. Chem. Soc.* **1936**, *58*, 1063.

William's First Synthesis



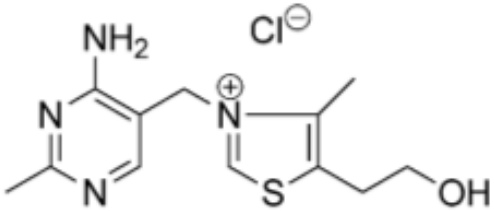
vitamin B_1 (thiamine)



Anion exchange to the chloride salt matched the elemental composition and UV absorption of the natural vitamin AND cures pigeons

Williams, R. R. *J. Am. Chem. Soc.* **1936**, *58*, 1504.

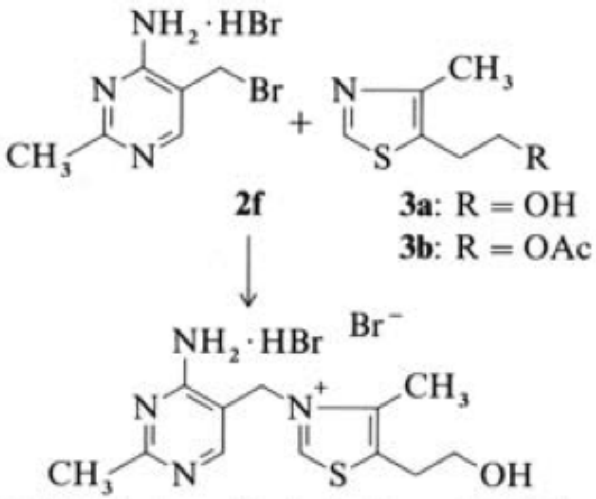
Industrial Synthesis



vitamin B_1 (thiamine)

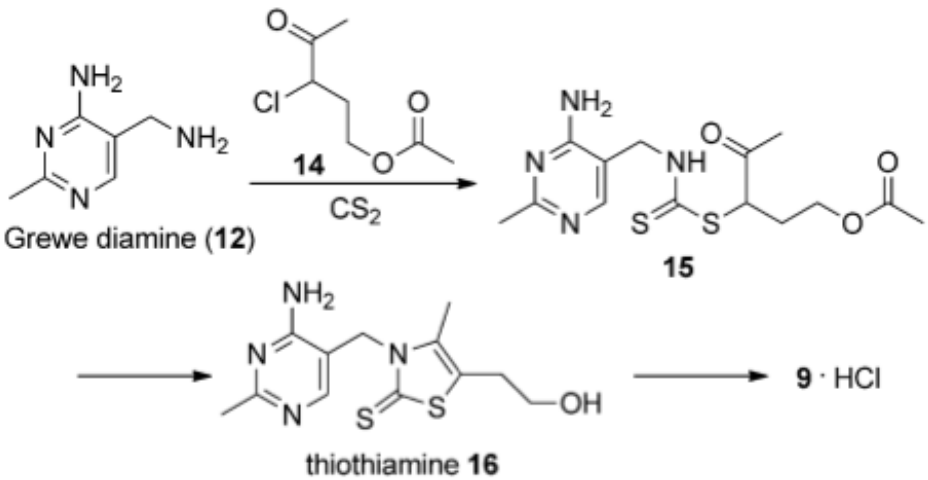
Due to low levels of B_1 in food, and its relative instability, sufficient quantities for fortified foods must be provided through chemical synthesis

1.7.1. Condensation of the Pyrimidine and Thiazole Rings



Thiamin bromide hydrobromide (**1d**)

1.7.2. Construction of the Thiazole Ring on a Preformed Pyrimidine Portion



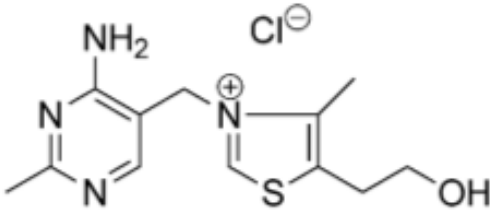
Grewe diamine (**12**)

thiothiamine **16**

All industrial syntheses proceed through Grewe diamine

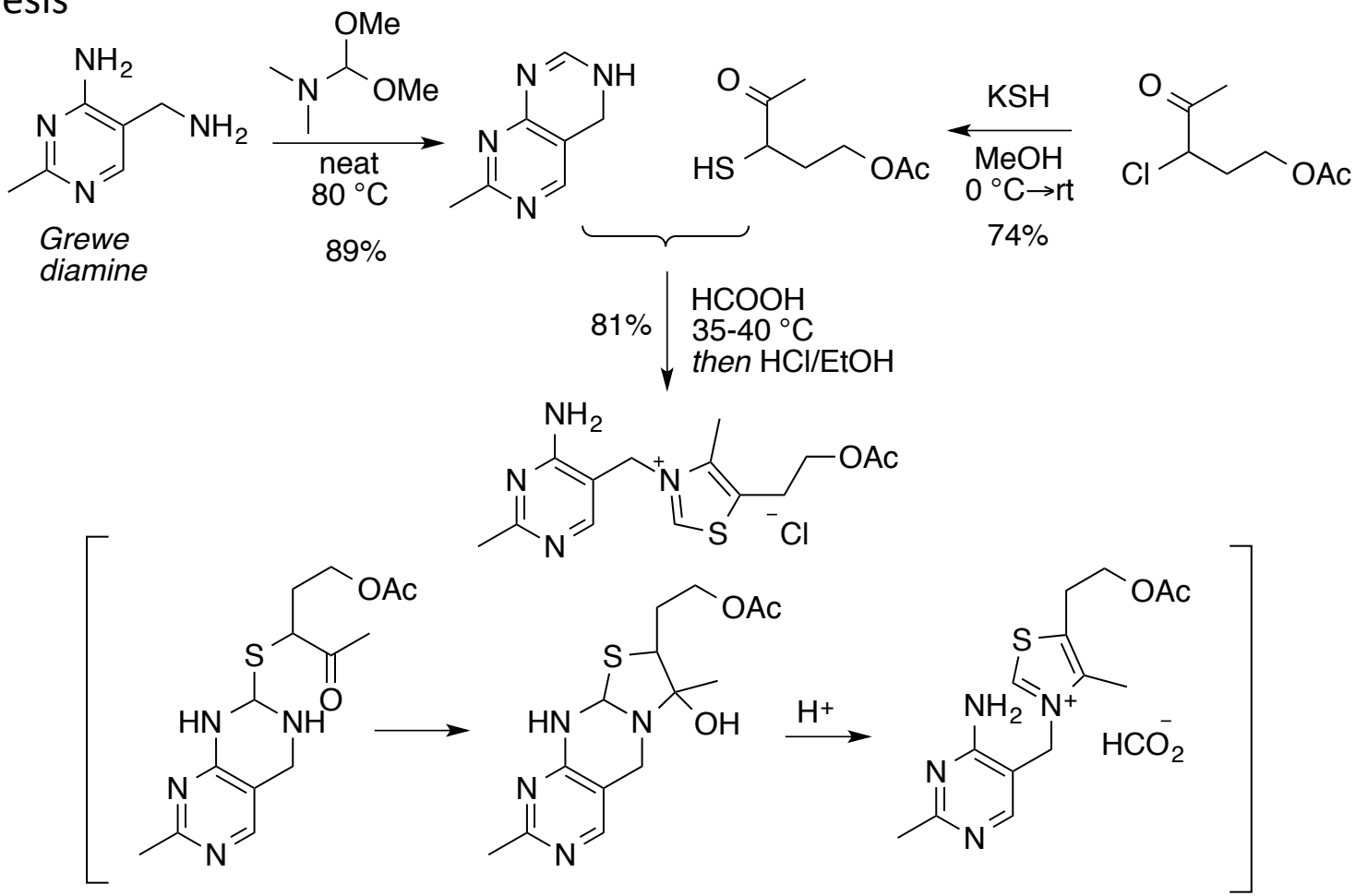
Moine, G.; Hohmann, H. –P.; Kurth, R.; Paust, J.; Hählein, W.; Pauling, H.; Weimann, B. –J; Kaeslet, B. *Ullman's Encyclopedia of Industrial Chemistry,* **2011**, Vitamins, 6. B Vitamins.

Hoffmann-La Roche Synthesis



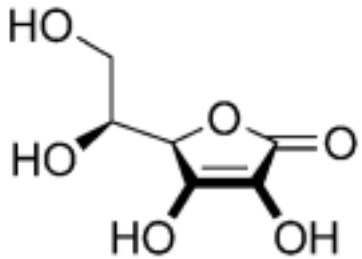
vitamin B_1 (thiamine)

Due to low levels of B_1 in food, and its relative instability, sufficient quantities to fortify foods must be provided through chemical synthesis



Contant, P.; Forzy, L.; Hengartner, U.; Moine, G. *Helv. Chimica Acta.* **1990**, *73*, 1300.

Vitamin C (Ascorbic Acid)



vitamin C (L-ascorbic acid)

Structure

- Exists as 2 bioactive forms (ascorbic acid & dehydroascorbic acid)
- Water soluble (lost in cooking)
- Easily oxidized upon storage
- Unstable to air & light
- Heat stable (pasteurization)

Storage in Body

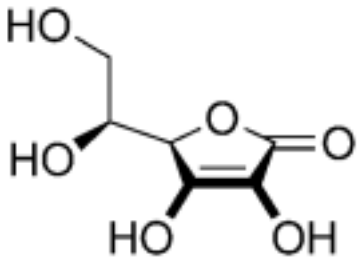
- Total body pool = ~1500 mg
- $t_{1/2}$ ~ 20 days
- Recommended daily intake 60 mg

Sources

- Most organisms can synthesize it from glucose
- Plants = fruits! (citrus, strawberries), potatoes, leafy greens
- Animal = only liver
- Often supplemented in soft drinks, cereal, flour, meat (curing)

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Vitamin C Biology



vitamin C (L-ascorbic acid)

Key Roles

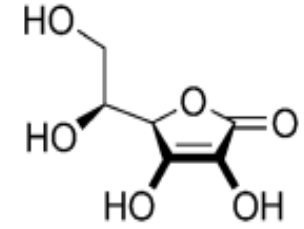
- Antioxidant (source of 1 and 2 e^-)
  (scavenges free radicals/ROS)
- Cofactor for oxygenases
  (direct reducant or reduces metal cofactor)
- Required in the biosynthesis of adrenaline,
  collagen, carnitine, tyrosine, and many neurotransmitters
- Important to cell-mediated immune response

Deficiency

- Common in extended travel in 15th-19th centuries
  (significant losses of soldiers and sailors)
- Uncommon in developed world due to
  fortification of foods
- Requires a very poor diet for an extended period of time
  (100-160 days)

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Scurvy Decimates Ships Crews



vitamin C (L-ascorbic acid)

1600 to 1800s

Age of exploration leads to extended time at sea for sailors, stressful living conditions resulted in a new disease that would kill entire crews.

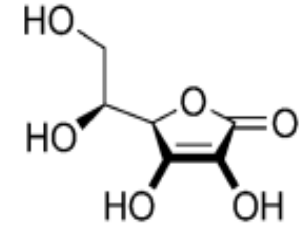
"The first symptom is pain in the whole body…purple spots being to cover the body… then the gums become so swollen that teeth cannot be brought together… and finally, they die all of a sudden, while talking."

- Chaplain, Spanish 1602 exploration of California

Scurvy persisted as a problem from 200 years for crews for all over the world

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 259.; Images from: *British Med. J.* **2001**, 322, 1544.; *BMJ Case Rep.* published online 20 March 2009, doi:10.1136/bcr.07.2008.0462

“Discovery” of Vitamin C



vitamin C (L-ascorbic acid)

Early 18th Century

Fruit and leafy greens were recognized to cure scurvy (usually due to landing on shore)

First attempts to find storable concentrate to cure disease included feeding sulfuric acid (scruvy was through to be an ‘alkaline disease’)

1746-1752: James Lind (ship surgeon, physician)

First controlled study of clinical nutrition

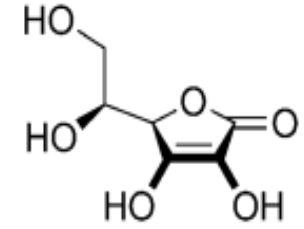
Systematically test known remedies for scurvy on a group of 12 sailors:

- 1.1 L hard apple cider
- 25 drops sulfuric acid in water × 3
- 18 mL vinegar × 3
- 0.3 L sea water
- 4 mL ‘medicinal paste’
- 2 oranges + 1 lemon

Supply ran out after only 6 days, however both men were sufficiently cured to care for others

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 259.

Lind's 'A Treatise of the Scurvy'



vitamin C (L-ascorbic acid)

1752: Published by James Lind (physician)

First to argue that any source of acid is insufficient, but that something in citrus was far superior

Sulfuric acid did result in cleaner mouths, but did not treat other symptoms

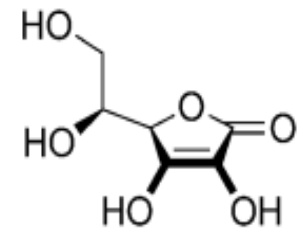
Postulated an interesting theory on the cause of scurvy:

"…the principle and main predisposing cause is the manifest and obvious air quality, namely its moisture. Scury is unknown in dry places."

Lind's reinvigorated the search for a stable concentrate to treat scurvy

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 259.

Search for the Cure



vitamin C (L-ascorbic acid)

1784: Thomas Trotter (navel surgeon)

Tested crystalline citric acid, but was not active against disease

1795: British Navy Supplies Lemon Juice

Changed courses for frequent resupply, over 1.6 million gallons issued over the next 20 years

1830: Lecture at teaching hospital in London

First statement of scurvy being a purely "chemical disease"

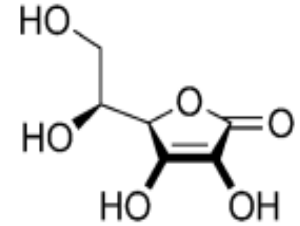
1845: Great Potato Famine

Diminished access to potatoes (a dietary staple) lead to large increases in scurvy in the general population

Land scurvy exists?

Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 259.

Alternative Theories Abound



vitamin C (L-ascorbic acid)

1850: Justus von Leibig (organic chemist)

Theorized that only protein (nitrogen-containing components) were nutritive, and deficiency diseases are due to a lack of protein

Widely accepted without evidence

1850s: "Potassium" Theory

Advances in analytical chemistry showed that diets of sailors were deficient in potassium, while soldier usually had an increased potassium intake

1861-1863: Potassium Fails to Treat Scurvy

Potassium supplementation was ineffective at treating scurvy in US soldiers (Civil War) and Canadian lumberjacks

1900s: Germ Theory Gains Traction

Evidence for the germ theory (and success of vaccines) for other diseases revitalized the idea that bacteria was the cause of scurvy

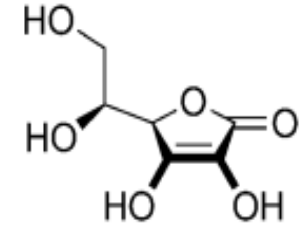
Carpenter, K. J. *Ann. Nutr. Metab.* **2012**, *61*, 259.

EXPERIMENTAL STUDIES RELATING TO SHIP-BERI-BERI AND SCURVY.

By AXEL HOLST, M.D.,
Professor of Hygiene,
and THEODOR FRÖLICH, M.D.,
University of Christiania.

(Plates XVII and XVIII.)

II. ON THE ETIOLOGY OF SCURVY.



vitamin C (L-ascorbic acid)

Desired to establish a beriberi disease model in mammals by feeding guinea pigs a refined and unrefined grain diet:

- pronounced hemorrhages in the hind legs & lower jaw
- fragile bones
- looseness and discolorization (green-grey) of the molar teeth
- swollen and bleeding gums

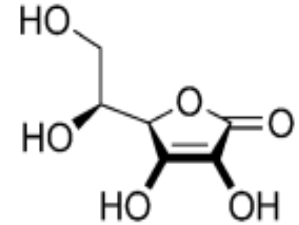
} Matches human scurvy!

→ disease is reversed by addition of lemons, cabbage, fresh potatoes into diet

First laboratory model of scurvy

Holst, A.; Frölich, T. *I. J. Hyg.* **1907**, *7*, 634.

Identification of "Reducing Factor"



vitamin C (L-ascorbic acid)

1928: Albert Szent-Györgyi (Hungarian Scientist)

First isolation using a rapid *in vitro* screening using a chemical titrations (reduction of iodine to iodide)

1. purified peroxidase $\xrightarrow[\text{Guaicacum}]{H_2O_2}$ **Blue-Green**

2. press juice (turnip) $\xrightarrow[\text{Guaicacum}]{H_2O_2}$ No color

3. press juice (turnip) $\xrightarrow[\text{Guaicacum}]{H_2O_2}$ No color $\xrightarrow{H_2O_2}$ **Blue-Green**

First quantities of H_2O_2 are consumed by a different component

4. press juice (turnip) $\xrightarrow[\text{Starch}]{I_2}$ Colorless

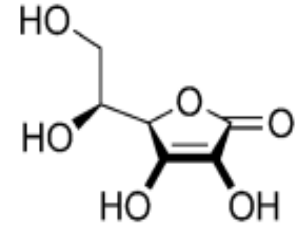
5. press juice (turnip) $\xrightarrow{H_2O_2}$ $\xrightarrow[\text{Starch}]{I_2}$ **Blue**

An additional substrate within the peroxidase enzyme, termed the "reducing factor" or R.F., is very rapidly oxidized by external reactants

Analytical titration of amount of R.F. present in foods based on I_2 reduction

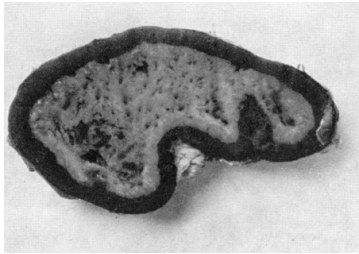
Szent-Györgyi, A. *J. Biochem.* **1928**, *22*, 1387.

Isolation of Hexuronic Acid

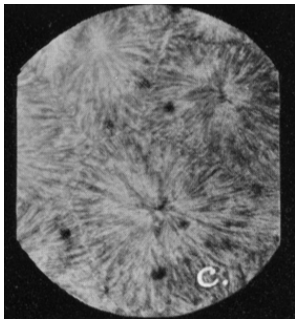


vitamin C (L-ascorbic acid)

1928: Albert Szent-Györgyi (Hungarian Scientist)



ox adrenal gland (1-3 kg) → over 15 steps → crystalline R.F. (300 mg)



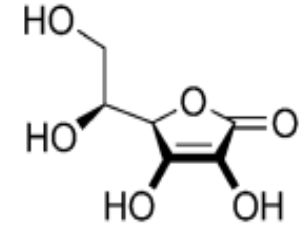
Properties:

- Air sensitive, water soluble
- Crystallizes from MeOH/ pet ether (polymorphic, m.p. 175-198 °C)
- Molecular weight of 178 by acidimetric & iodometric titration
- Elemental analysis for $C_6H_8O_6$ (M.W. 176)
  found: C 40.7 H 4.7 theorectical: C 40.9 H 4.5
- Positive qualitative tests for carbohydrate and reducing agent
- Iodine titration confirms hexuronic acid loses 2H atoms in oxidation of I_2
- Oxidized hexuronic acid can be reduced by animal tissue (kidney, liver, muscle)

Further applied similar isolation method to oranges and cabbage to yield identical hexuronic acid

Szent-Györgyi, A. *J. Biochem.* **1928**, *22*, 1387.

Hexuronic Acid as the Antiscorbutic Factor



vitamin C (L-ascorbic acid)

1932: Charles Glen King (Univ. of Pittsburgh)

The recrystallized substance from lemon juice ("vitamin C") that cures guinea pigs of scurvy matches hexuronic acid.

1932: Albert Szent-Györgyi (Hungarian Scientist)

Confirms King's result with guinea pig assay 2 weeks later.

basal diet ONLY (oats, bran, butter, salt, water) → all 9 died within 20-34 d; severe scurvy

basal diet + 1 mL lemon juice → all 8 healthy at 55 d; mild symptoms of scurvy

basal diet + 1 mg hexuronic acid → all 10 healthy at 55 d; NO symptoms of scurvy

Svirbely, J. L.; Szent-Györgyi, A. *Nature* **1932**, *129*, 576.
King, C. G.; Waugh, W. A. *Science* **1932**, *75*, 357.

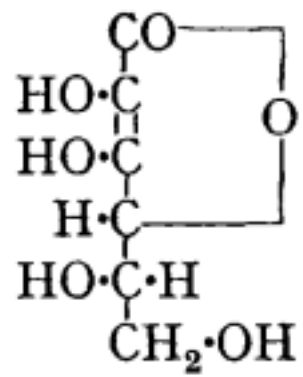
Structural Assignment

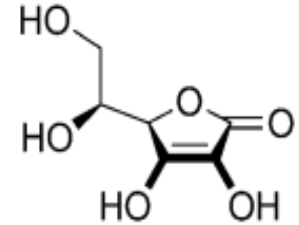
1933: Norman Haworth

Proposed furanose structure X for ascorbic acid

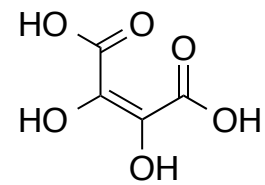
- not really a hexuronic acid ($C_6H_8O_6$) = ascorbic acid
- weak organic acid gives salt of type $C_6H_7O_6M$
- qualitative tests are positive for ketone (hydrazone) and enol (ferric chloride) but not for aldehyde
- Reaction with I_2 and UV absorption similar to dihydroxylmaleic acid

- Ozonolysis of tetramethyl-ascorbic acid confirms structure

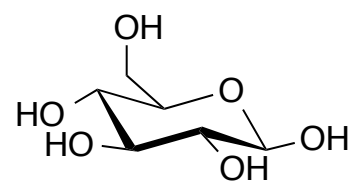




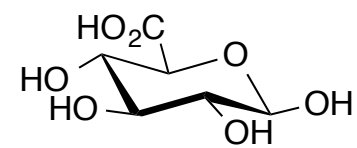
vitamin C (L-ascorbic acid)



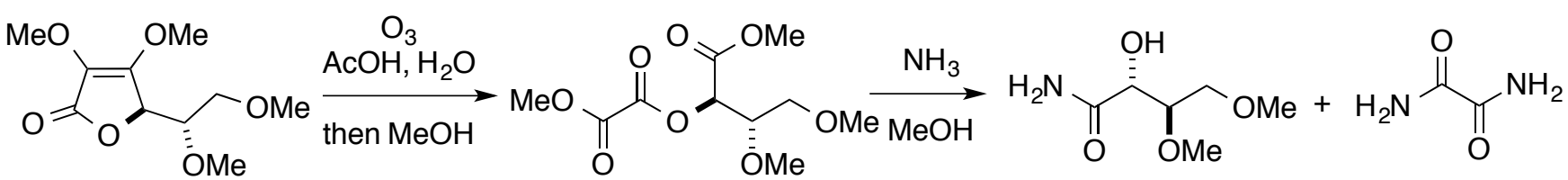
dihydroxymaleic acid



β–D-glucose
$C_6H_{12}O_6$



β–D-glucuronic acid
$C_6H_{10}O_7$



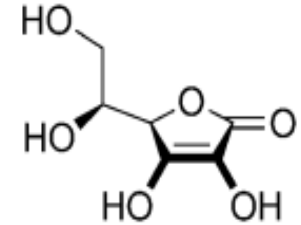
tetramethyl ascorbic acid

(+ minor epimization)

oxamide

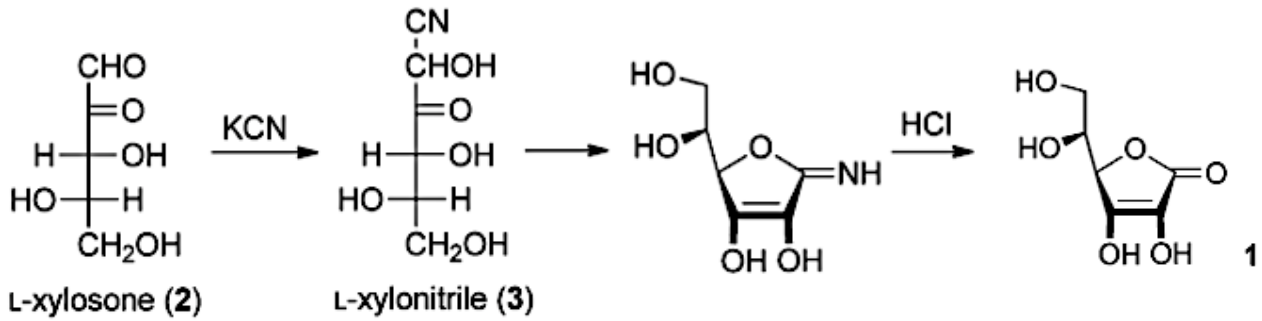
Haworth, W. N.; Hirst, E L.; Reynolds, R. J. W. *Nature* **1932**, 129, 576.
Herbert, R. W.; Hirst, E. L.; Percival, E. G. C.; Reynolds, R. J. W.; Smith, F. *J. Chem. Soc.* **1933**, 1270.

First Total Syntheses of Vitamin C



vitamin C (L-ascorbic acid)

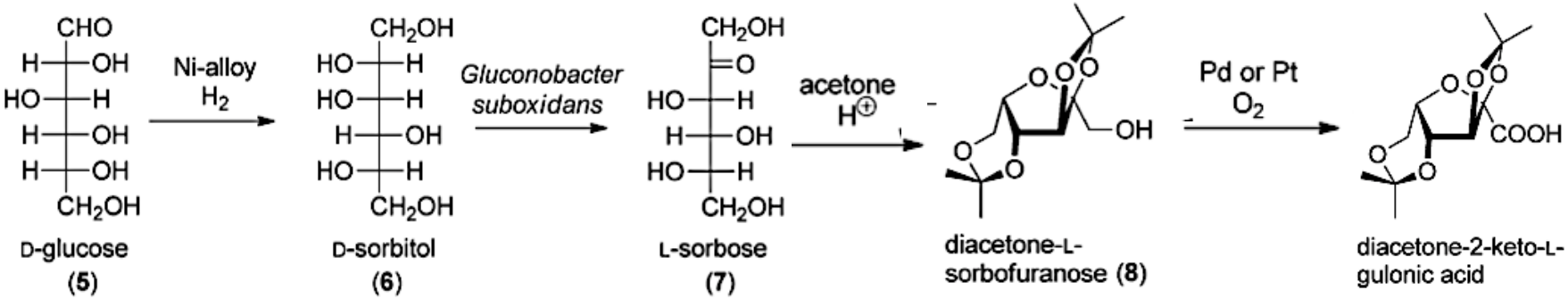
1933: Normal Haworth



Scheme 1. Synthesis of L-ascorbic acid from xylosone.

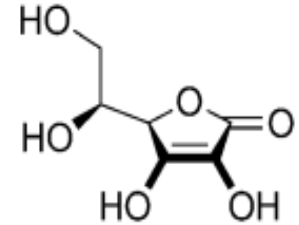
Never commercialized due to high cost of starting sugar

1933: Reichstein-Grüssner Process: First Vitamin Industrial Synthesis



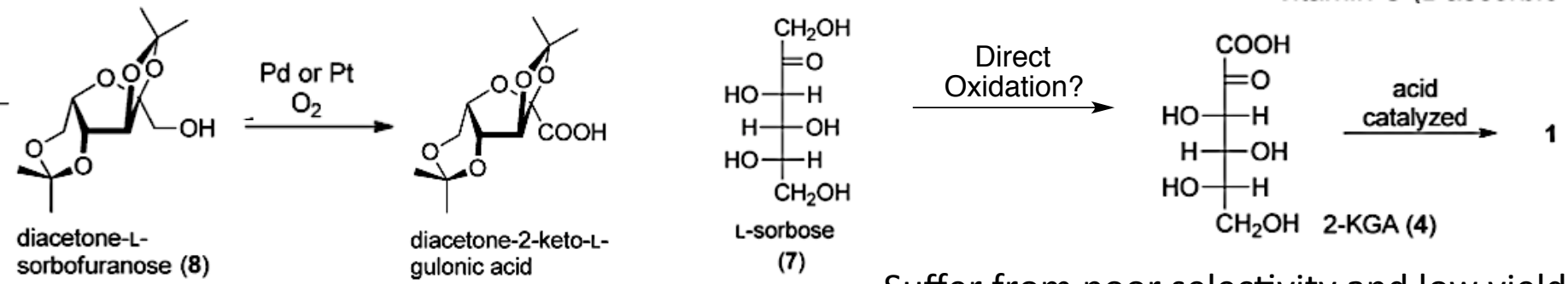
Haworth, W. N.; Hirst, E. L. *J. Chem. Soc. Ind.* **1933,** *52*, 645.; Reichstein, T.; Grüssner, A.; Oppenauer, R. *Helv. Chim. Acta* **1933**, *16*, 561.
Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

Improvements in Vitamin C Synthesis



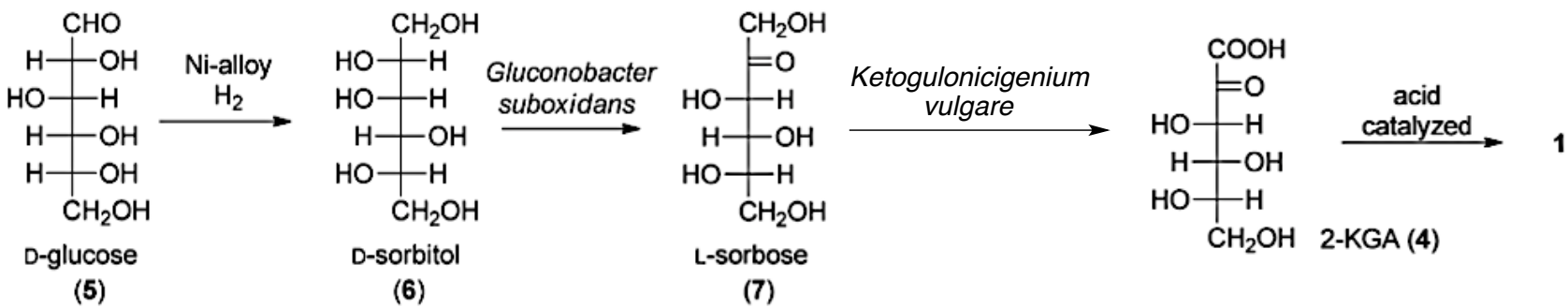
vitamin C (L-ascorbic acid)

Direct Oxidation



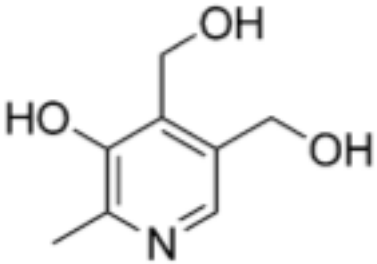
Suffer from poor selectivity and low yield

Two-Stage Microbial Fermentation



Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

Vitamin B_6 (Pyridoxine)



vitamin B_6 (pyridoxine)

Structure

- Exists as 6 "vitamers" (pyridoxine, pyridoxal, pyridoxamine + phosphates)
- All forms interconvert *in vivo*
- Water soluble (loss in cooking)
- Light & heat sensitive, loss upon storage

Storage in Body

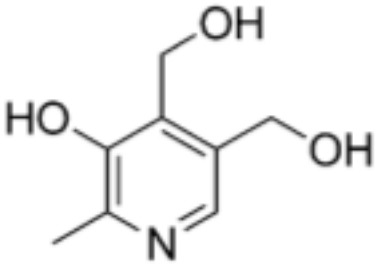
- Total body pool ~40-150 mg
- $t_{1/2}$ ~33
- Requirement is increased w/ high protein intake
- Located in liver, brain, kidney, spleen, and muscles

Sources (widely distributed)

- Animal = meat, eggs (less stable)
- Plants = unrefined cereal, grain, veggies (more stable)
- Supplemention often unnecessary

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Vitamin B_6 Biology



vitamin B_6 (pyridoxine)

Key Roles

- Coenzyme for >60 known enzymes
- Key coenzyme for protein metabolism and amino acid synthesis
- Needed for the synthesis of neurotransmitters

Discovery

- Cures rat acrodynia
- 5 groups simultaneously elucidated structure

Deficiency

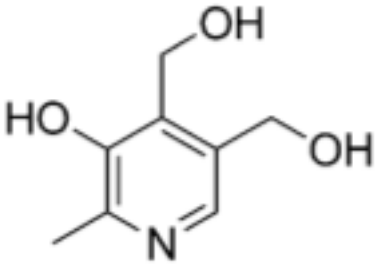
- Uncommon due to wide distribute in a variety of foods

Symptoms of Deficiency

- weakness, irritability, nervousness, susceptibility to noise
- weight loss
- depression, loss of responsibility
- insomnia

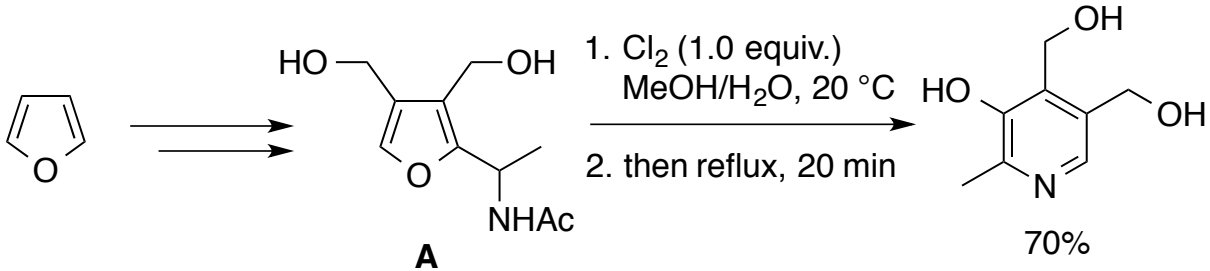
G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Group Question

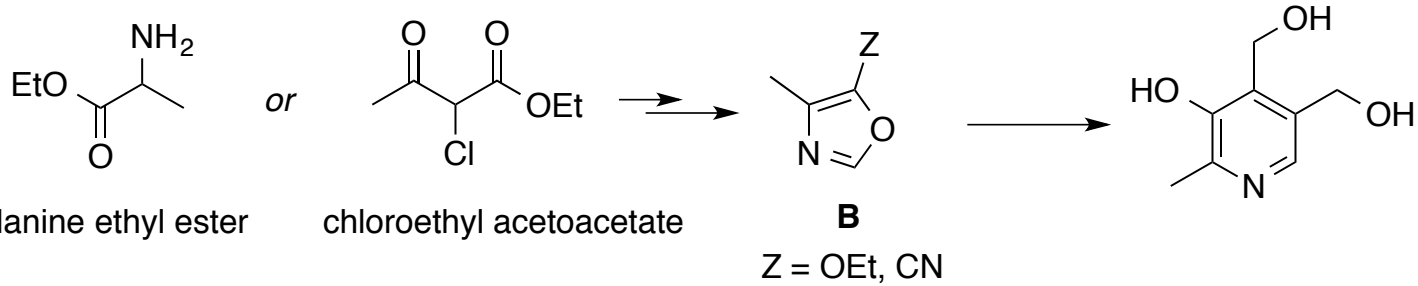


vitamin B_6 (pyridoxine)

1. Industrial synthesis of pyridoxine in the 1950s started from furan-derivative **A**
   a.) Propose a synthesis of **A** starting from furan
   b.) Draw a mechanism for final step producing vitamin B_6



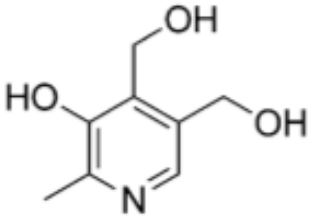
2. Industrial pyridoxine synthesis is now is exclusively produced from oxazole derivatives **B**. Propose a synthesis from **B** to pyridoxine that is more concise than above.



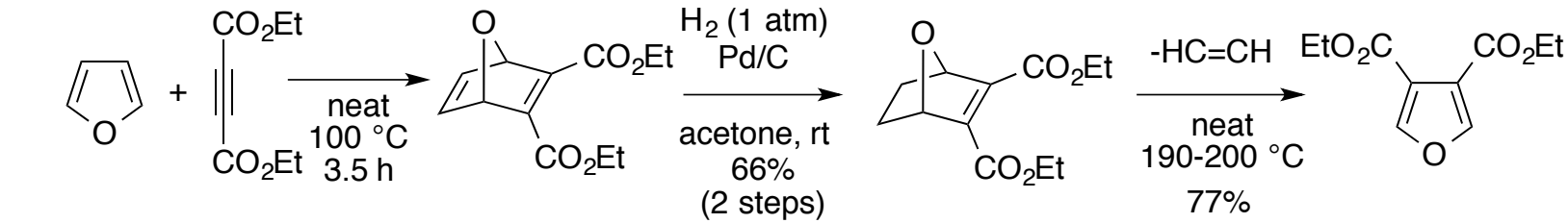
Moine, G.; Hohmann, H. –P.; Kurth, R.; Paust, J.; Hählein, W.; Pauling, H.; Weimann, B. –J; Kaeslet, B. *Ullman's Encyclopedia of Industrial Chemistry,* **2011**, Vitamins, 6. B Vitamins.

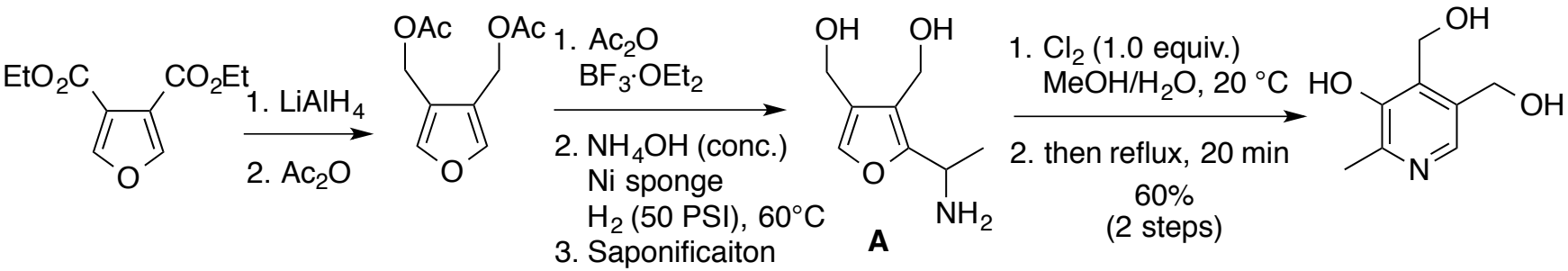
Pyridoxine From Furans

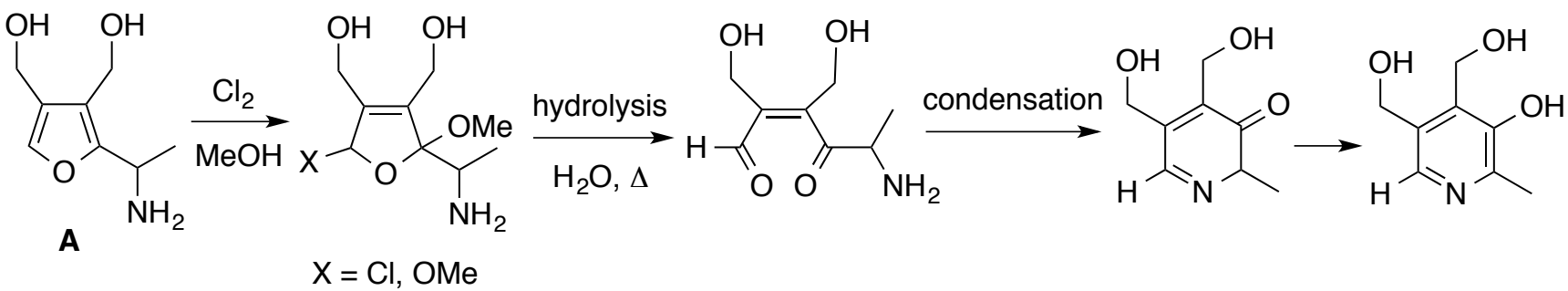
1. a.) Propose a synthesis of **A** starting from furan
   b.) Draw a mechanism for final step producing vitamin B_6



vitamin B_6 (pyridoxine)

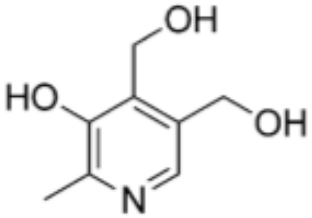






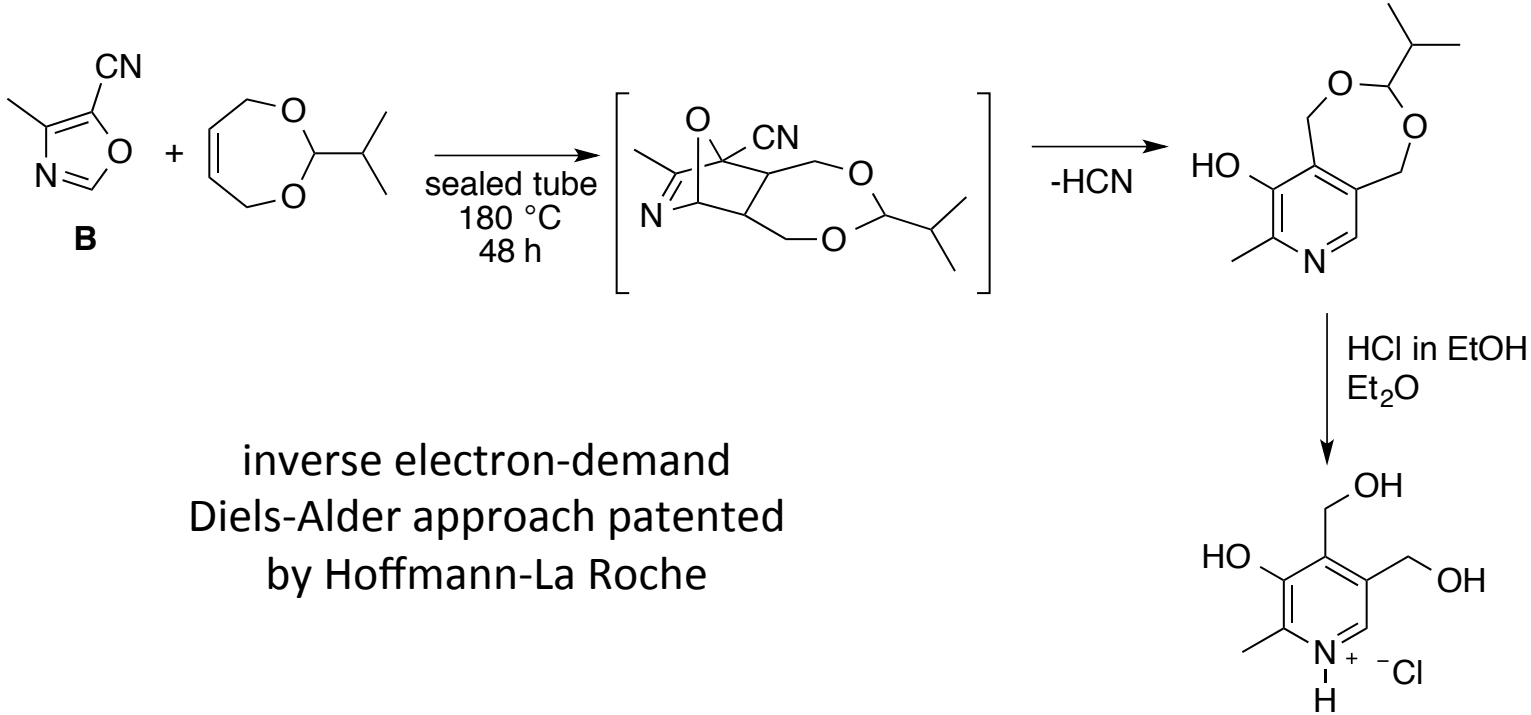
Clauson-Kaas, N.; Elming, N.; Tyle, Z. *Acta Chemica Scandinavica* **1955**, *9*, 1.
McDoweel, W. B.; Moes, H. U.S. Patent 2,855,407. June 17, 1958.
Elmin, N.; Nielsen, J.; Konrad, N.; Clauson-Kaas, F. W. U.S. Patent 2,839,539. June 17, 1958.

Pyridoxine From Oxazoles



vitamin B_6 (pyridoxine)

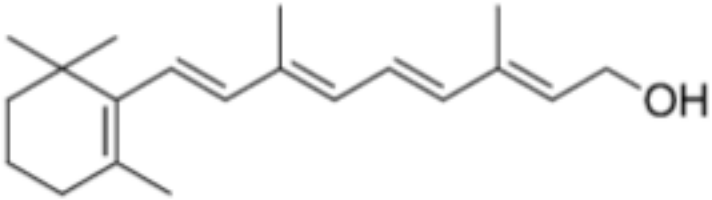
2. Industrial pyridoxine synthesis is now is exclusively produced from oxazole derivatives **B**. Propose a synthesis from **B** to pyridoxine that is more concise than above.



inverse electron-demand
Diels-Alder approach patented
by Hoffmann-La Roche

Kimel, W.; Leimgruber, W. U.S. Patent 3,250,778. May, 10, 1966.

Vitamin A (Retinol)



vitamin A (retinol)

Structure

- Exists as 3 bioactive forms (rentinol, retinal, & rentinoate)
- Exists as the *all trans* form
- Absorbs light at 325 nm
- Unstable to air & light
- Heat stable (pasteurization)

Sources

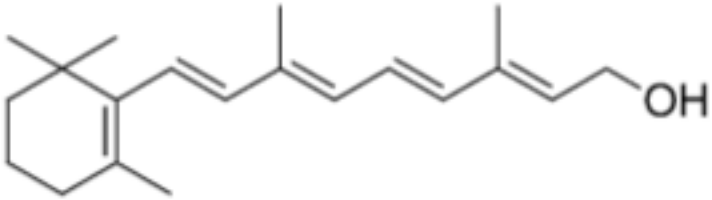
- Animal = eggs, fish oil, dairy, liver
- Plants = leafy green, yellow/orange veggies
- Often supplemented in margarine

Storage in Body

- Primarily in the liver (~90%)
- Secondary = kidneys, lungs, adrenals, retina
- Absorption requires dietary fat (bile salts)
- Circulation regulated by homeostasis

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Vitamin A Biology



vitamin A (retinol)

Key Roles

- Pigment for vision of color & dim light
- Immune response (humoral & cell-mediated)
- Epithelial cell differentiation/maintainence
- Reproduction and embryo growth

Symptoms of Deficiency

- Night blindness/complete blindness
- Drying out of epithelial tissues (cornea, intestine, skin, muscous membranes)

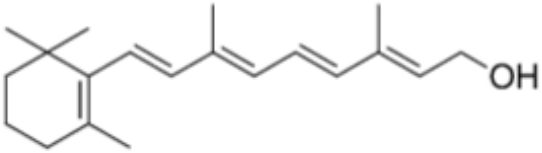
Deficiency

- Common in developing countries (estimated 190 million pre-school children)
- Responsible for large proportion of children mortality & blindness
- Also caused by diets deficient in Zn, protein, or fat

Vitamin A supplementation is one of the most successful interventions for child mortality (on par with immunizations and bed nets for malaria)

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

"Discovery" of Vitamin A



vitamin A (retinol)

Early 1800s

Health disparities between rich and poor people of Paris gets attention

1816: Francois Magendie (physiologist)

Feeding studies with dogs concluded that a non-nitrogen containing diet of sugar water leads to weight loss and eye lesions

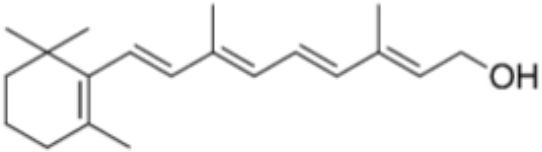
1810s: Charles-Michel Billard (pediatrican)

Similar eye lesions were observed on abandoned infants

Eye lesions in the poor population may be due to diet deficiency

Semba, R. D. *Ann. Nutr. Metab.* **2012**, *61*, 192.

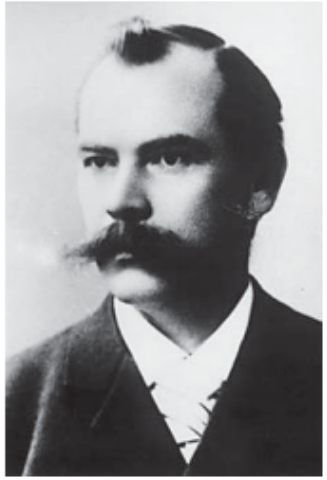
Mice & Milk



vitamin A (retinol)

1881: Nicolai Lunin (doctoral student)

Mice can live on milk but not the individual components (proteins, fats, carbohydrates, sat, water)

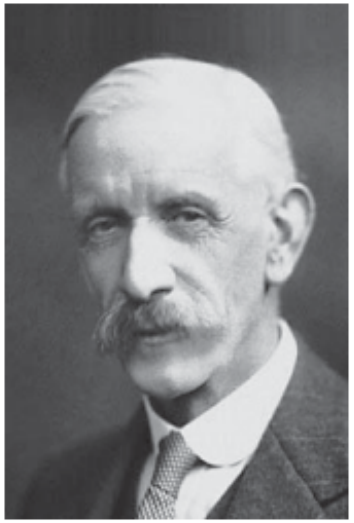


1887: Carl A. Socin (Lunin's coworker)

Mice fed egg yolk with Fe lived longer than Fe alone
First to speculate the unknown dietary factor was fat soluble

1909: Paul Knapp (ophthalmologist)

Milk prevents eye lesions in mice



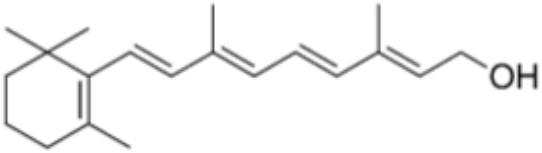
1906-1912: Frederick Gowland Hopkins (biochemist)

First to suggest "…no animal can live upon a mixture of pure protein, far, and carbohydrate, and even inorganic materials is supplied the animal still cannot fluorish"

Instead proposed that "unsuspected dietetic factors" account for deficiencies disease such as rickets & scurvy

Semba, R. D. *Ann. Nutr. Metab.* **2012**, *61*, 192.

Hopkins 1912 Landmark Study

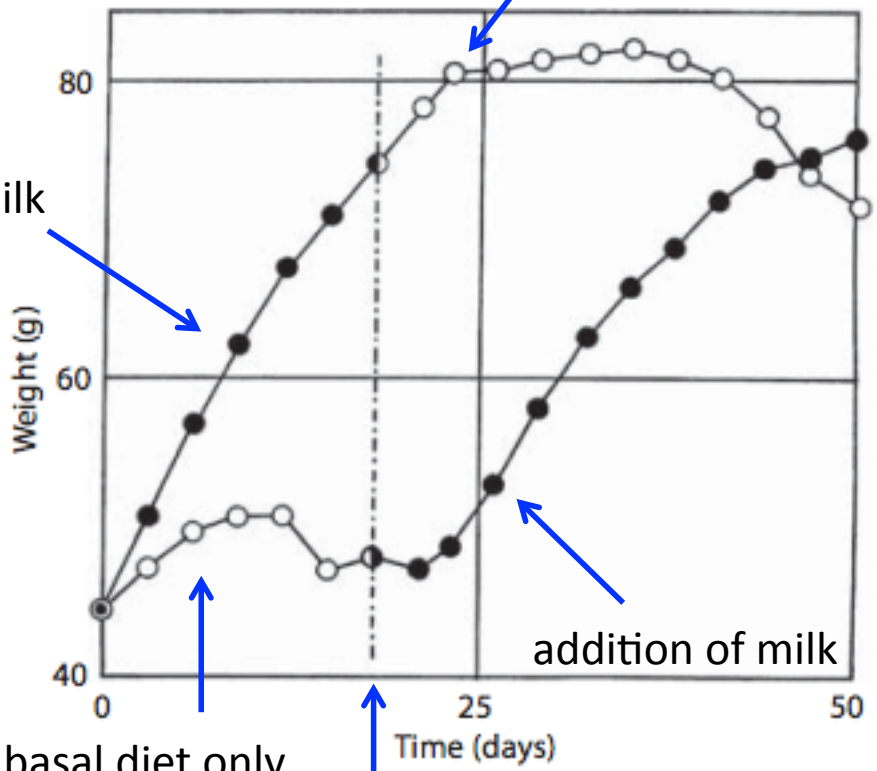


vitamin A (retinol)

Studied the unknown factor in milk that supported growth in young rats

exclusion of milk

basal diet + milk



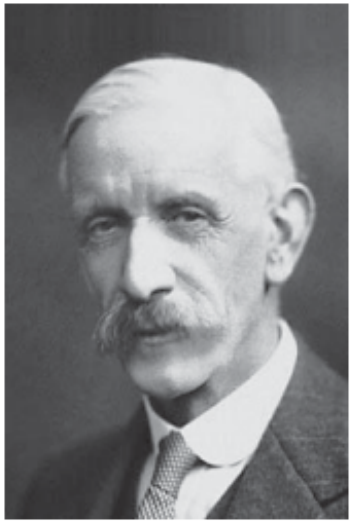
addition of milk

Basal diet = protein, starch, sugar cane, lard, and minerals

basal diet only

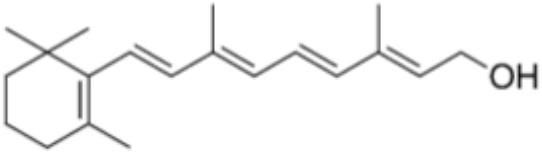
change in diet

1929 Nobel Prize in Physiology



Semba, R. D. *Ann. Nutr. Metab.* **2012**, *61*, 192.

Extractions of "Dietetic Factor"



vitamin A (retinol)

1911: Wilhelm Stepp (medical student)

"milk bread" (flour + milk) extracted by alcohol-ether did not sustain mice. Reintroduction of extracts did.

1913: Elmer McCollum

Not all fats are equal for the growth of rats, tested ether extracts from a variety of sources

butter or egg yolk extracts = growth
lard or olive oil extracts = death

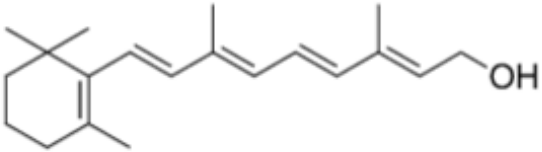
Terms this "fat-soluble A" in distinction to" water-soluble B"

McCollum claims he was the first to discover Vitamin A which is debatable

Extracts at this time still contained what we know to be Vitamins A, D, and E

Semba, R. D. *Ann. Nutr. Metab.* **2012**, *61*, 192.

Isolation of Vitamin A



vitamin A (retinol)

1931: Paul Karrer (chemist)

First to use fractional crystallization (no distillation) to obtain high purity Vitamin A

Proposed correct molecular formula $C_{20}H_{30}O$
with elemental analysis
found: C 83.84 H 10.56 theorectical: C 83.86 H 10.56

1937: Harry Holmes and Ruth Corbet (chemists)

fish liver oil (25 g) $\xrightarrow[\text{i-PrOH, rt, 1.5 h}]{\text{KOH}}$ "jelly-like" soap $\xrightarrow[\text{EtOH/H}_2\text{O, Pet. Ether ×4}]{\text{extraction}}$ crude Vit A (in MeOH) under N_2 $\xrightarrow[\text{filter ppt}]{\text{ice-salt bath}}$ $\xrightarrow[\text{filter ppt}]{\text{CO}_2\text{ (s) refigerator}}$ enriched Vit A (in MeOH) under N_2

enriched Vit A (in MeOH) under N_2 $\xrightarrow{\text{conc. to 1/2 vol.}}$ $\xrightarrow[\text{filter ppt}]{\text{CO}_2\text{ (s) refigerator}}$ $\xrightarrow{\text{add 1\% H}_2\text{O}}$ $\xrightarrow{\text{CO}_2\text{ (s) refigerator}}$ $\xrightarrow[\text{through CO}_2\text{(s)}]{\text{cold filtration}}$

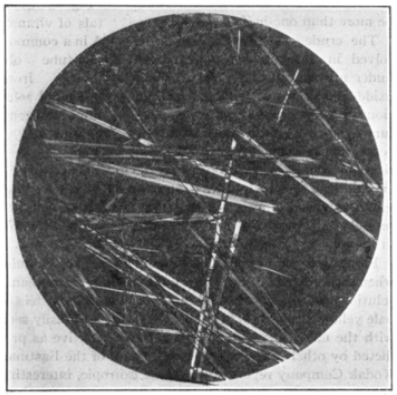
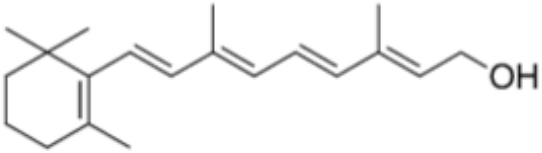


Fig. 2.—Yellow crystals of vitamin A photographed through the microscope by polarized light.

Karrer, P.; Morf, R.; Schöpp, K. *Helv. Chim. Acta.* **1931**, *14*, 1036.
Homes, H. N.; Corbet, R. E. *J. Am. Chem. Soc.* **1937**, *59*, 2042.

Characterization of Vitamin A



vitamin A (retinol)

1937: Harry Holmes and Ruth Corbet (chemist)

Elemental Analysis matched Kerrer's formula $C_{20}H_{30}O$
found: C 83.28 H 10.44 theorectical: C 83.86 H 10.56

m.p. was identical for multiple starting fish oils (7.5-8 °C)

Molecular weight determinations by freezing point depression
attempts in benzene were "met with disaster"
more successful in cyclohexane
determined 290, 295, 300 (avg. 294) --- (actual MW: 286)

Highest extinction coefficient reported to date (2100)

Very low doses needed in rat feeding studies

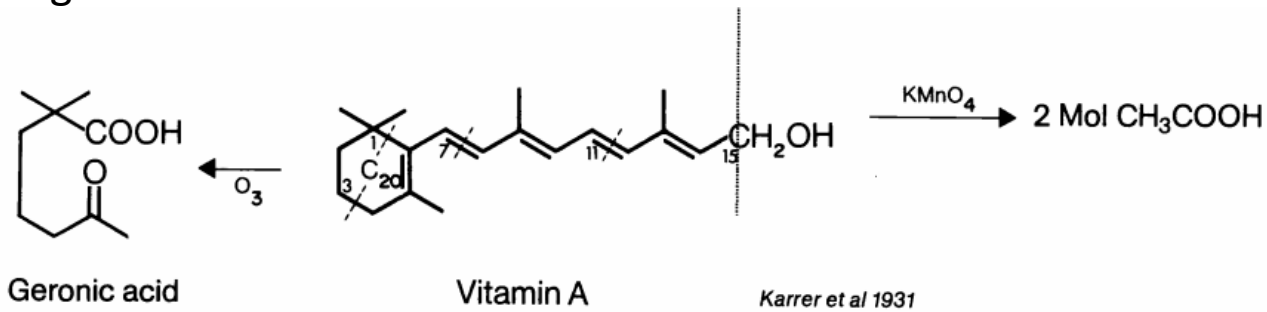
Fig. 1.—Crystals of vitamin A in a common test-tube obtained from methanol solution at low temperatures.

Holmes, H. N.; Corbet, R. E. *J. Am. Chem. Soc.* **1937**, *59*, 2042.

Structural Elucidation & First Synthesis

1931: Karrer

Oxidative degradation used to determine Vitamin A structure

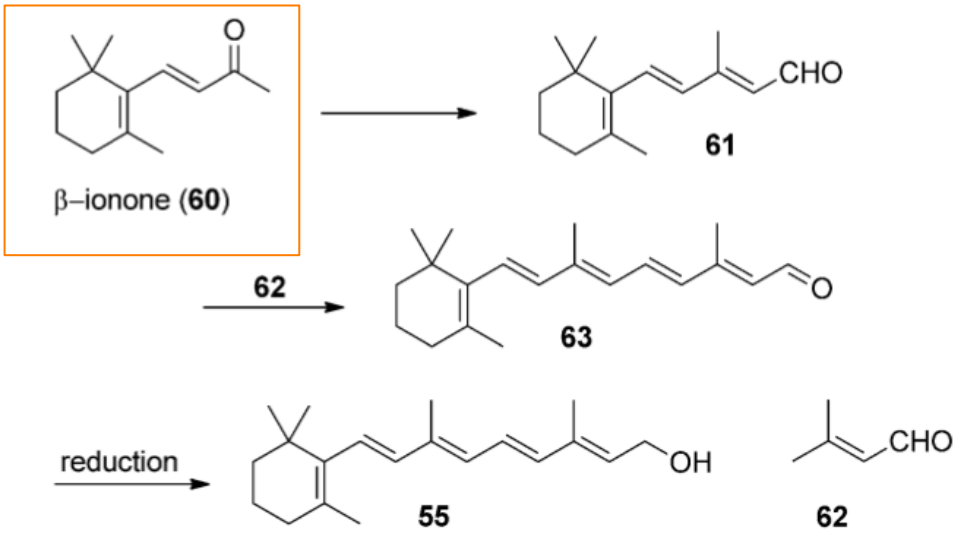


Karrer et al 1931

1937: Kuhn & Morris

Resulting yellow oil only had a 7.5% Vitamin A content.

β-ionone serves as the starting material for efficient future syntheses

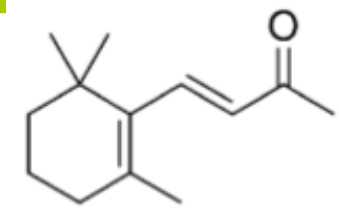


Scheme 30. First synthesis of vitamin A by Kuhn and Morris.

Kuhn, R.; Morris, C. J. O. R. *Ber. Dtsch. Chem. Ges.*. **1937**, *70*, 853.

Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

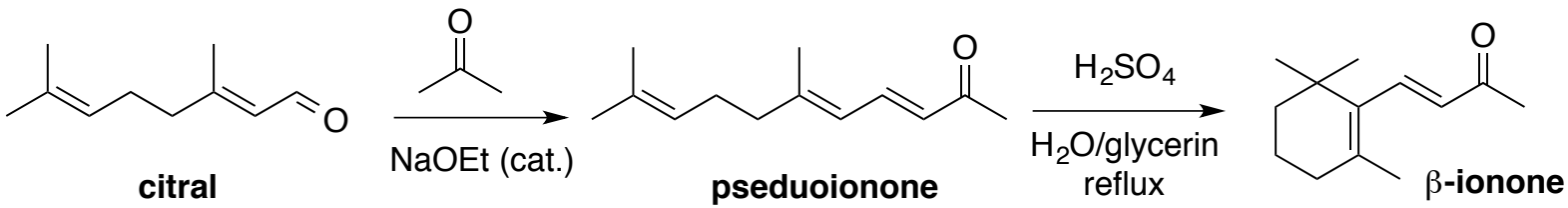
β-Ionone Synthesis



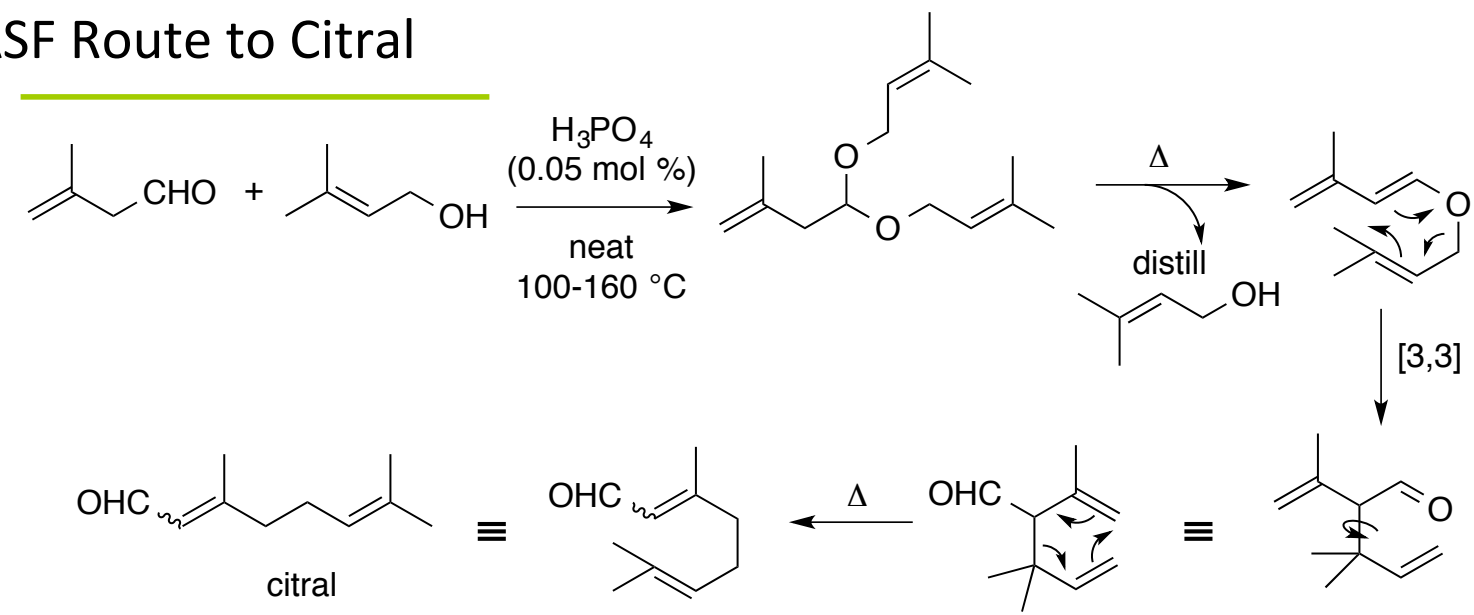
β–ionone (**60**)

Synthesis from citral (lemongrass)

19th century process as a component of perfume, however lemongrass oil was not an economical source for Vitamin A production



BASF Route to Citral



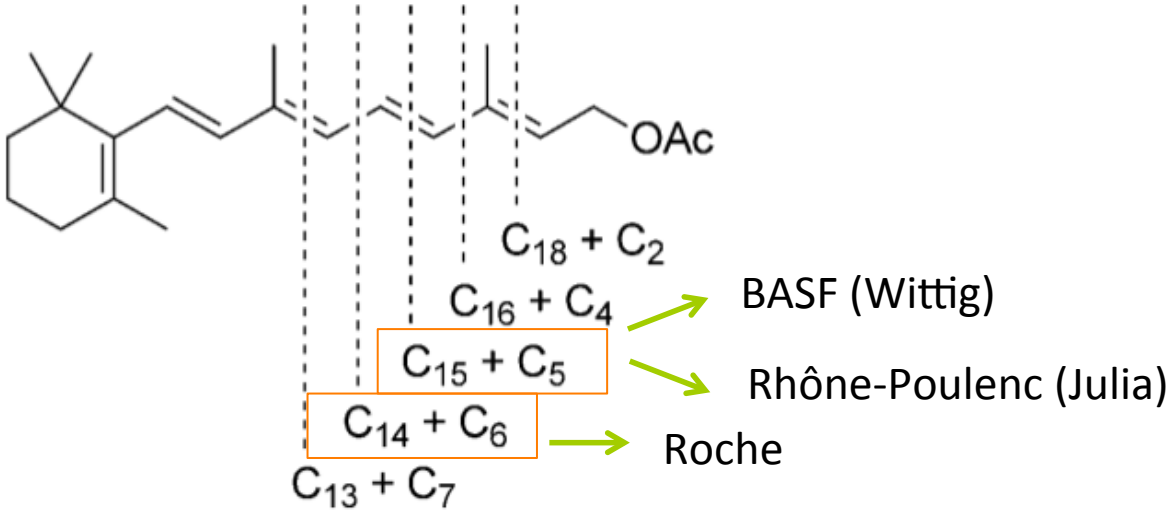
Krauth, W. U.S. Patent 652,969, July 3, 1900.

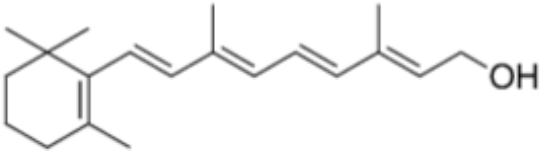
Nissen, A.; Rebafka, W.; Werner, A. U.S. Patent 4,288,636, Sep. 8, 1981.

Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

Industrial Synthesis

3 Different Approaches





vitamin A (retinol)

Figure 15. Otto Isler (left) with his co-worker Gody Ryser (source: Roche Historical Archive).

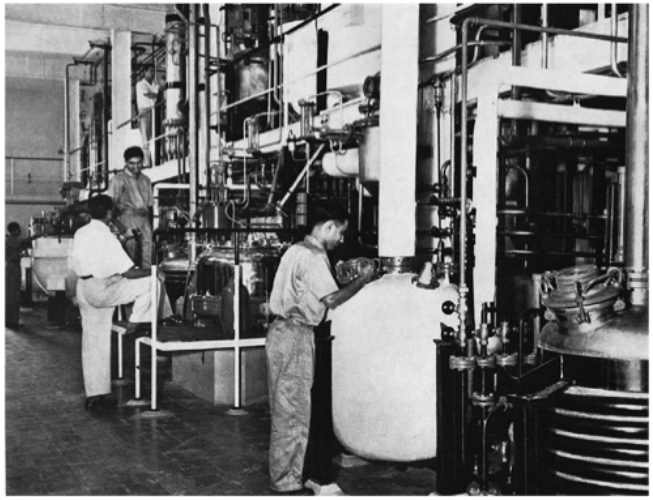
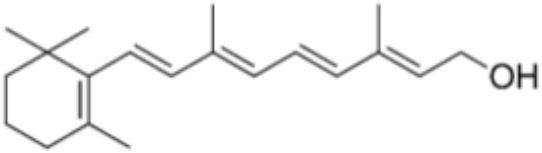


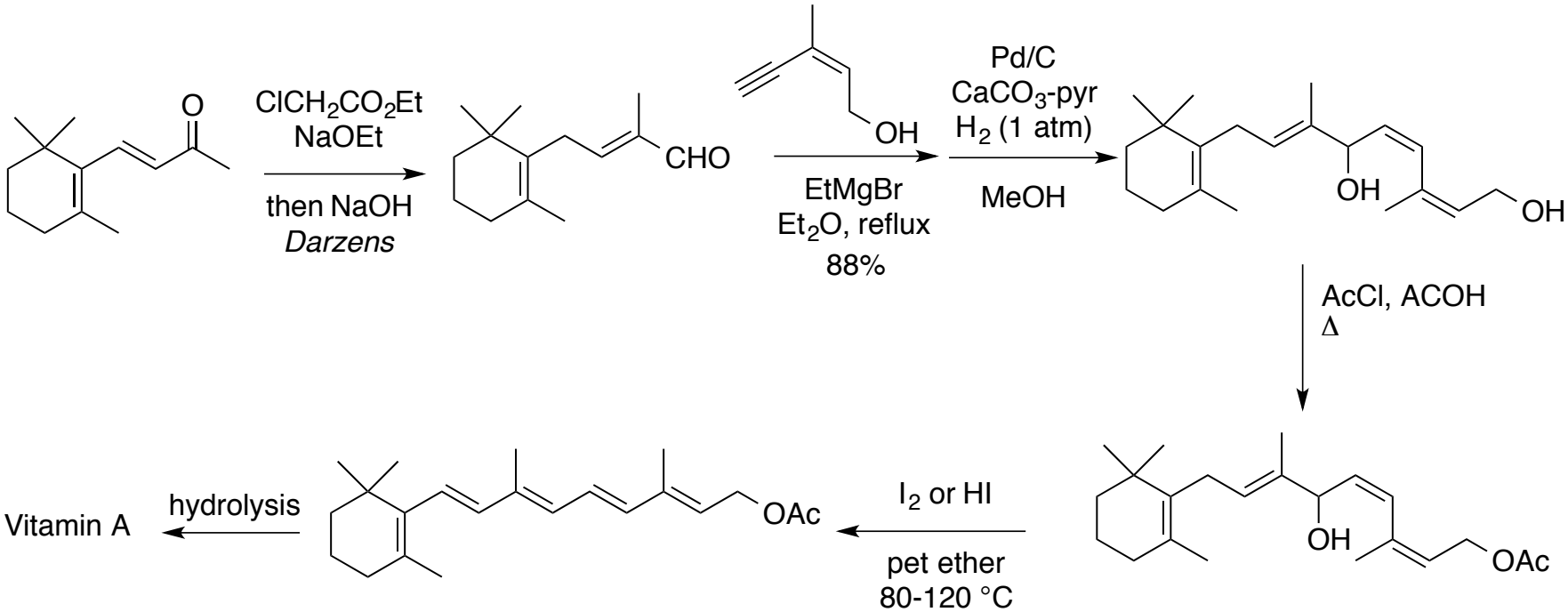
Figure 16. Early production of vitamin A at Roche Nutley, USA (source: Roche Historical Archive).

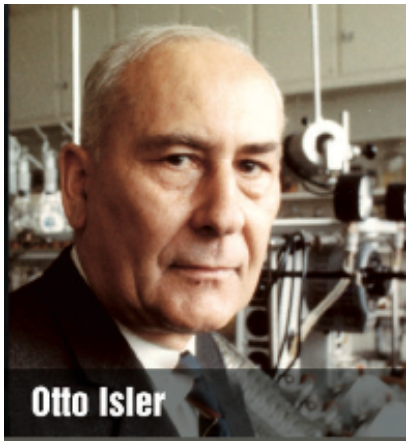
Semba, R. D. *Ann. Nutr. Metab.* **2012**, *61*, 192.

Roche C_{14} + C_6 Synthesis



vitamin A (retinol)

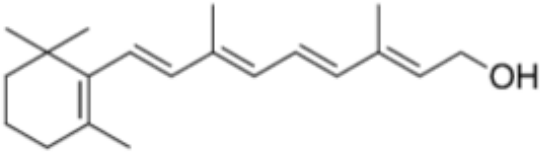




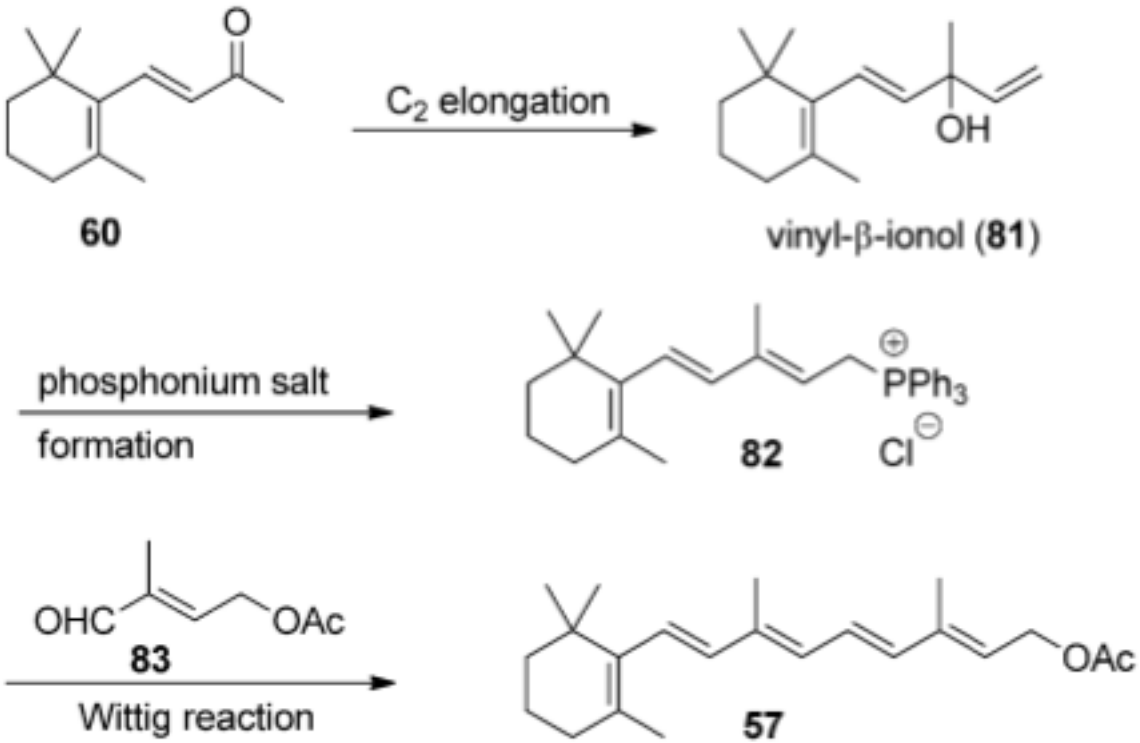
Isler, O. US Patent 2,451,739. Oct. 19, 948.

Isler, O.; Huber, W.; Ronco, A.; Kofler, M. *Helv. Chimica Acta* **1947**, *30*, 1911.

BASF C_{15} + C_5 Synthesis



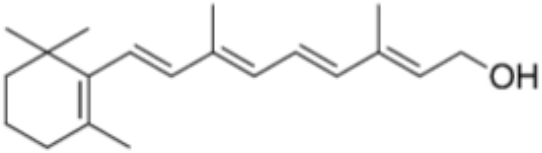
vitamin A (retinol)



eme 37. BASF route to vitamin A by using a Wittig reaction.

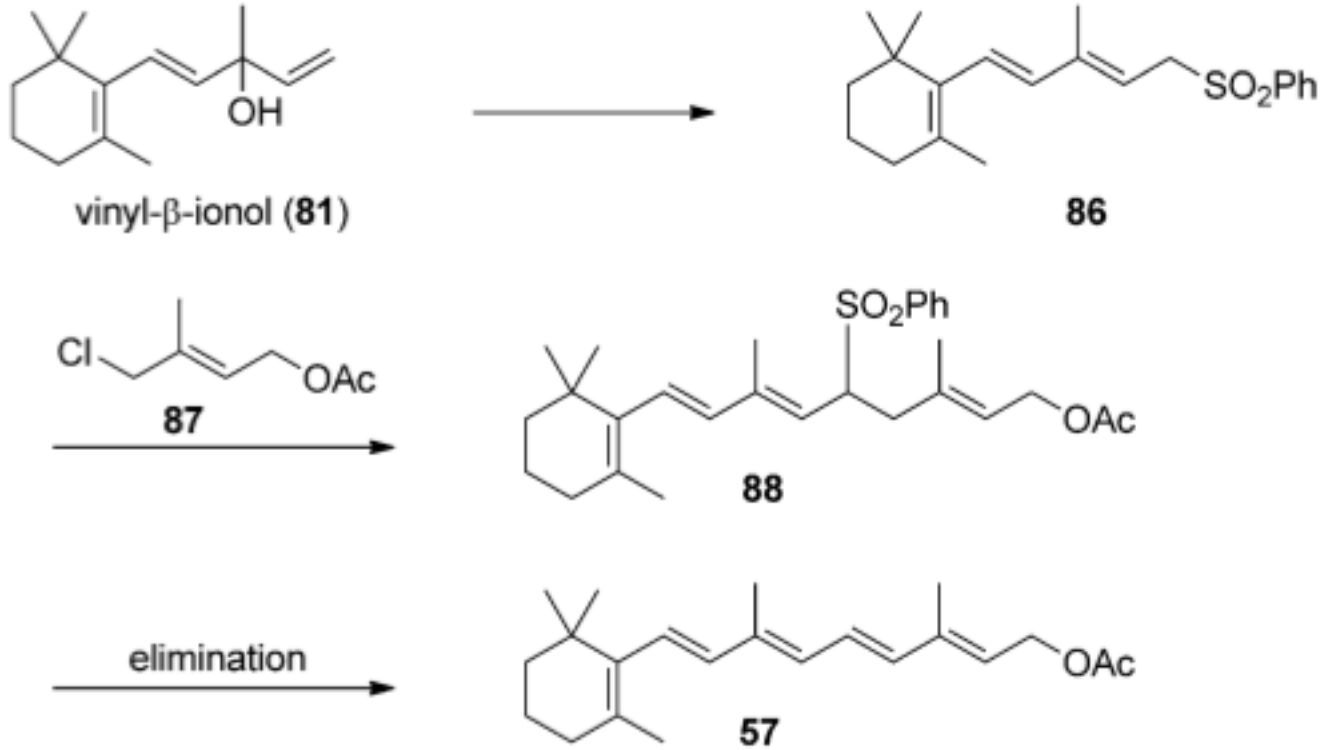
Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

Rhône-Poulenc C_{15} + C_5 Synthesis



vitamin A (retinol)

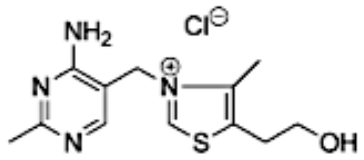
3 Different Approaches



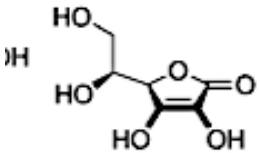
Scheme 39. Rhône–Poulenc synthesis of vitamin A.

Eggersdorfer, M.; Laudert, D.; Létinois, U.; McClymont, T.; Medlock, J.; Netscher, T.; Bonrath, W. *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

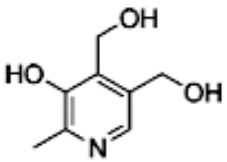
Summary



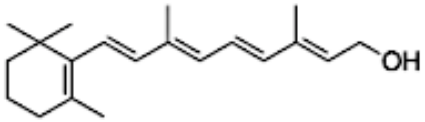
vitamin B_1 (thiamine)



vitamin C (L-ascorbic acid)



vitamin B_6 (pyridoxine)



vitamin A (retinol)

- The history vitamins is demonstrates the importance of acute scientific observation, serendipity, and global market demand in the advancement of science
- Chemical synthesis, through the optimization for very efficient syntheses, has had a huge impact on global health by supplying vitamins in large quantity
- Discovery and production of vitamins was one of the earliest demonstrations that organic synthesis can have huge impact on discoveries of human health and disease

References

- Historical/General Reviews:

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Ann. Nutr. Metab. **2012**, *61*, 185-265. (Special Issue on 100 years of Vitamin Research)

- Chemistry-Oriented Reviews:

Eggersdorfer, M. *et. al.* "One Hundred Years of Vitamins – A Success Story of the Natural Science" *Angew, Chem. Int. Ed.* **2012**, *51*, 12960.

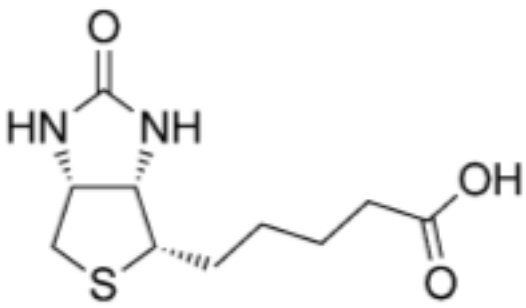
Moine, G.; Hohmann, H. –P.; Kurth, R.; Paust, J.; Hählein, W.; Pauling, H.; Weimann, B. –J; Kaeslet, B. *Ullman's Encyclopedia of Industrial Chemistry,* **2011**. (Contains individual chapters on each class of vitamins).

Isler, O. "History and Industrial Application fo Carotenoids and Vitamin A" *Pure Appl. Chem.* **1979**, *51*, 447..

Bonrath, W.; Netscher, T. "Catalytic Processes in Vitamin Synthesis and Production." *App. Cat. A.* **2005**, *280*, 55.

Bonrath, W.; Eggersdorfer, M.; Netscher, T. "Catalysis in the Industrial Preparation of Vitamins and Neutrachemicals."*Cat. Today* **2007**, *121*, 45.

Biotin (Vitamin B_7, Vitamin H)



vitamin B_7 (vitamin H, biotin)

Structure

- Covalently linked as amide to enzyme *in vivo*
- Only shown stereoisomer is active
- Heat sensitive (loss in cooking, curing)
- Light sensitive, loss upon storage

Storage in Body

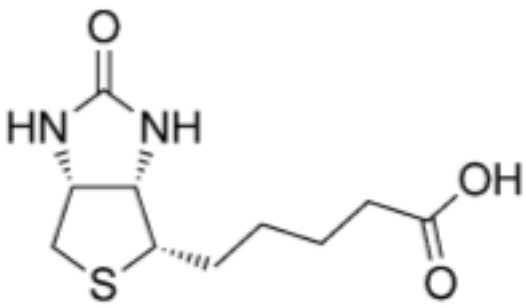
- Requirement intake estimated at 50-300 µg
- Dispersed throughout body

Sources (widely distributed)

- Animal = organ meats, egg yolk, yeast
- Plants = cauliflower, legumes, mushrooms, nuts
- Largest source is likely intestinal bacteria

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**

Biotin Biology



vitamin B_7 (vitamin H, biotin)

Key Roles

- Coenzyme for carboxylases (covalently linked)
- Activated bicarbonate to transfer CO_2
- Needed for the synthesis of neurotransmitters

Deficiency

- Uncommon due to wide distribute in a variety of foods
- Occurs with excessive diet of raw eggs (30% of calories from raw egg)
- Populations with long-term antibiotic use
- Alcoholics, pregnant women, and infants

Symptoms of Deficiency

- loss of appetite
- alopecia
- seborrhoeic dermatitis (red flaky irritated skin)
- Potential link to SIDS

G. F. Combs, Jr. *The Vitamins, Fundamental Aspects in Nutrition and Health, 4th ed.,* Academic Press, London, **2012.**