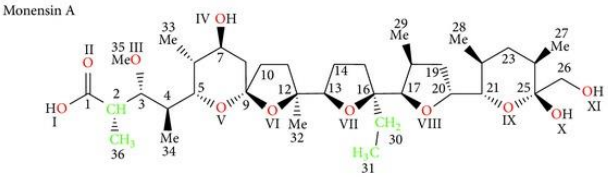
TECHNISCHE UNIVERSITÄT DARMSTADT

Monensin

JONATHAN ACHTER, JANA ANTON, FRAN BACIC TOPLEK, SARAH BRANDNER
Technische Universität Darmstadt

Abstract

Monensin is an ionophore that belongs to the group of polyether antibiotics and has the ability to disrupt ionic gradients by selectively complexing and thereby transporting monovalent cations across biological membranes. The broad range of applications has led to commercial use in agriculture, especially against coccidiosis and for increasing the digestion efficiency in ruminants. It is now one of the best-studied examples of antibiotics based on a polyether structure and it is still being discussed in current research projects.

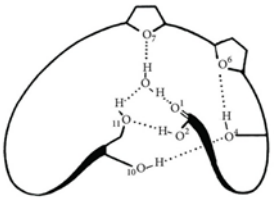


I. History

Monensin was first reported and isolated from Streptomyces cinnamonensis by Agtarap et al. in 1967 at the Lilly Research Laboratories. Its discovery led to an increasing interest in polyether antibiotics. It became the first of five ionophores known at that time whose structure was decoded by mass spectrometry.
The first total synthesis of monensin, by Yoshito Kishi, succeeded in 1979 and second in the following year by W. Clark Still. In 1971, the drug was introduced into the US and is used in the beef and poultry industries to rid poultry of coccidiosis. However, its use as a feed additive was prohibited in 2006 in the EU. Since 2013, monensin may be used as a drug, not as an additive, in the beef and dairy industries under the name Kexxtone®.

II. Structure and properties

[i]Monensin A occurs as a monohydrate which has a water molecule complexed inside. Intramolecular hydrogen bonds between the initial carboxylic group and two opposite hydroxyl groups maintain monensin molecules in a pseudocyclic conformation. Monensin possesses an exterior alkyl backbone that confers lipophilic properties, while an internal, oxygen-rich cavity is responsible for the molecule's ability to bind metal ions.



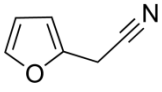
The carbon-scaffold forms ring structures: two tetrahydropyran (six-membered) and three tetrahydrofuran (five-membered) rings, each containing an oxygen atom.
Another structural property of monensin is its heterocyclic spiro center.

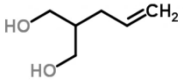
Characterization of monensin A

Classification	Polyether
Molecular weight	670.9 g/mol
Molecular formula	$C_{36}H_{62}O_{11}$
Melting point	103 – 105°C (monohydrate)
Specific rotation	+47,7°
Asymmetric centers	17
Homologues	Monensin A,B and C

III. Production / synthesis

Monensin is produced in vivo by Streptomyces cinnamonensis as a natural defense against competing bacteria. Monensin presents a formidable challenge to synthetic chemists as it possesses 17 asymmetric centers on a backbone of only 26 carbon atoms.
Although its total synthesis has been described (e.g., Kishi et al., 1979), the high complexity of monensin makes an extraction from the bacterium the most economical procedure for its production.
The total synthesis has 56 steps and a yield of only 0.26%. The chemical precursors are 2-allyl-1,3-propanediol and 2-(furan-2-yl)acetonitrile.
The method used for synthesizing monensin is based on the principle of "absolute asymmetric synthesis". Molecules are constructed out of prefabricated building blocks in the correct conformation, aiming for higher yields of the desired enantiomer. New stereocenters are also introduced. Using this method, monensin is assembled in two parts, a larger right side and a smaller left one.
The penultimate step is connecting the left and the right halves of monensin, which are independently generated, in an Aldol-condensation. The two halves' keto end groups (C7/ C8) are linked by eliminating a water molecule. The C7 atom is favored over the C1 atom, because it is more reactive. For catalyzing this step, Yoshito Kishi's group used *i*Pr_2NMgBr (Hauser base) and THF to coordinate it at a temperature of – 78°C. Thus, they were able to isolate the molecule in the right conformation at a ratio of 8:1. Due to the low temperature required for a high yield of the correct enantiomer, the reaction is very solw.
One of the most difficult steps is the last one: the connection of the spiro center. This is due to a characteristic feature of spiro compounds; they open and close very easily. Therefore, the conditions for forming the right conformation must be optimal in the last step of synthesis.
The biosynthesis in a cell culture of Streptomyces cinnamonensis involves a complex medium containing, among other components, glucose, soybean oil, and grit. Cultivation is carried out for a week at a temperature of 30°C and under constant aeration. Product isolation requires filtration, acidification to pH3, extraction with chloroform and purification with activated carbon. In this way, a few grams per liter of monensin are produced and isolated. For crystallization, azeotropic distillation is necessary.
In vivo, polyether backbones are assembled by modular polyketide synthases and are modified by two key enzymes, epoxidase and epoxide hydrolase, to generate the product. Precursors of the polyketide pathway are acetate, butyrate and propionate.

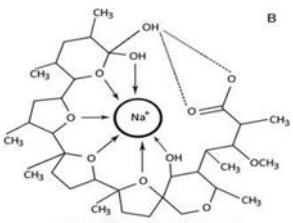




precursor in the total synthesis

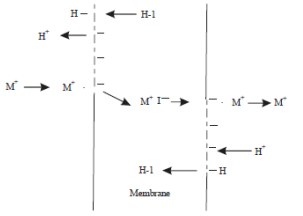
IV. Effects

[ii] Ionophores are a chemical species that catalyze the transport of certain ions across lipophilic membranes. They can be divided into two major groups. So called quasi ionophores, also referred to as channel formers, and carrier ionophores. In the latter case, guest species like alkali cations are brought into internal cavities of the molecule by polar ligating groups. These cyclic cation– ionophore complexes are soluble in lipophilic media and can diffuse through biological membranes, releasing the enclosed cation on the opposite side.

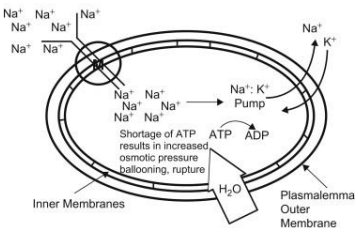


Monensin, as a monocarboxylic polyether, is characterized by its terminal carboxyl group, which is fully ionized at physiological pH. Head-to-tail hydrogen bonding stabilizes the molecule in a pseudo-cyclic structure with a size that is similar to that of a 17-membered ring. The formation of such three-dimensional inclusion complexes allows monensin to selectively complex monovalent alkali cations with the highest affinity being for sodium. The guests are coordinated by six nucleophilic oxygen atoms (ether oxygens and hydroxyl groups) at internal sites, so that the aliphatic residues are positioned outside and solubilize guest species in the nonpolar membrane.

[iii]The ionophoric activity of monensin is based on its function as a metal– proton antiporter. In vivo, the polyether antibiotic occurs in its anionic form, stabilized by the polar environment. Surrounded by metal cations, the ionophore binds and thereby initiates the formation of a cyclic cation– ionophore complex (abbreviated MonM (Mon^-M^+)) at the interface of the membrane. The carboxylate is not employed at this stage and remains deprotonated. The monensin– metal complex is neutral in charge, but has a dipole moment that is responsible for a longer passage time. When the lipophilic complex diffuses to the opposite side of the bilayer, it is again exposed to a polar environment, wherefore the ionophore releases the enclosed cation and reverts to the lower energy acyclic conformation. As diffusion cannot occur in the anionic form of monensin, Mon^- is first protonated (MonH). The electroneutral molecule then crosses the membrane, where it exchanges the proton for an alkali cation.



[iv]Most cells maintain a high intracellular potassium and a low intracellular sodium concentration. As a result, monensin facilitates the influx of sodium and the efflux of potassium. Due to membrane potential, the potassium gradient is greater than the sodium gradient, and protons accumulate inside the target cell.



The cell reacts to the disruption of its ion gradient by activating an ATP-dependent sodium pump called Na^+/K^+-ATPase that catalyzes the transport of sodium out of the intracellular medium in exchange for K^+. This gradual depletion of its energy storage means the cell eventually runs out of ATP at the expense of essential cellular processes such as nutrient absorption, culminating in cell death. Simultaneously, owing to the loss of the transmembrane electrical potential difference, an uncontrolled uptake of water takes place and swells up the target cell.

The same mechanisms are used by other polyether antibiotics such as salinomycin and ionomycin with the exception that they are selective for different ions.

Monensin shifts the microbial population of rumens, so that bacteria that produce acetate and butyrate are decreased, while propionate producing bacteria are increased. In addition to an antimicrobial application against gram-positive bacteria, monensin also has slightly antifungal, antiviral and antiparasitic effects, explaining the pharmacological response against coccidiosis, which is caused by coccidian protozoa (eukaryotic single-celled parasites).

It has been reported that monensin shows cardiovascular effects, although the mechanism of action is still unclear. It is believed that catecholamine is partially involved, because the effect of the ionophore can be suppressed by β-adrenergic inhibitors. Nonetheless, Ca^{2+} concentration seems to play a key role owing to its importance for muscular contraction and cardiac functionality.

Because of its low gastrointestinal resorption and high first-pass-metabolism, polyether antibiotics, such as monensin, can be added to fodder. Monensin is rapidly metabolized in the liver into products with fewer antimicrobial properties, indicating a reduction of biological activity via metabolism. It has been shown that oxidative metabolism occurs for instance by the protein superfamily cytochrome 450, which function as monooxygenases. Most of the metabolites result from O-demethylation or hydroxylation at the backbone.

Beside the ionophoric activities, it has also been proved that monensin A inhibits protein transport into the Golgi apparatus. Furthermore, it damages mitochondria of animal cells and slows down the process of endocytosis as well as intracellular transport by inducing a pH change.

Monensin also has an impact on external structures of the cell by reducing the secretion of molecules such as lamin, fibronectin and collagen, which are components of the extracellular matrix.

V. Use and applications

Monensin was formerly used in poultry and cattle breeding due to its positive impact on livestock husbandry, which led to increased economic profit. After reevaluation, the polyether antibiotic was prohibited in 2006 as a food additive in Europe in order to reduce the spread of antibiotic resistances.

Because of monensin's antibacterial, antifungal and antiparasitic properties, it can be used for the treatment of diseases caused by the protozoa coccidiosis, which can lead to life-threatening haemorrhagic inflammations. It is also used to treat histomoniasis, another parasitic infection.

Antibiotic activity has been shown against Micrococcus, Bacillus and Staphylococcus. Food metabolism of animals is also improved by the ionophore as it shifts the microbial population in favor of gram-negative bacteria. These prokaryotes produce copious amounts of propionic acid, a gluconeogenic precursor, thus growth is stimulated by means of increased energy storage.

Another benefit of monensin is its biological effectiveness in the prophylaxis of ketosis. Ketosis is a metabolic state of the body that occurs after long periods of a negative energy balance (low ingestions of carbohydrates) and is seen in cattle. As a consequence, the body produces large amounts of ketones, which accumulate in the blood and are used as an energy supply instead of blood glucose. Ketosis degrades fats as well as muscle and eventually leads to a fatty liver and disruption of the acid– base equilibrium. Absence of appetite then causes an insufficient food intake so that the animal remains in ketosis.

In 2013, monensin was introduced to the market again, this time as a prescription-only medicine called Kexxtone. Kexxtone is used for individual animals with an increased

risk of suffering from ketosis, for example in the perinatal period of dairy cattle. It is administered orally with the aid of a Bolus Gun.
The situation in the United States differs, as monensin is still used as a food additive for cattle and goats. In general, there is no application on humans because of toxicity issues. Typical symptoms of intoxication are diminished appetite, diarrhea and lethargy. Nevertheless, monensin is a current topic in cancer and malaria research. It has been reported that monensin enhances the effects of the immunotoxin 260F9-rRTA, which is used against breast cancer. Furthermore, it shows positive effects against resistant malaria mosquitos.

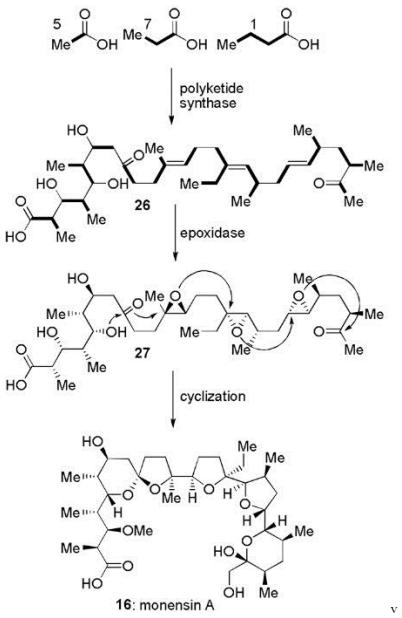
VI. Conclusion

The carboxylic ionophore monensin is a broad spectrum antibiotic with a wide range of target structures (bacterial, fungal, viral, even neoplastic and cardiovascular). Its current application is in veterinary medicine, where it is used to control coccidiosis and the efficiency of feed absorption. Due to toxicity issues, application in humans still fails. The group of polyether antibiotics is furthermore a current research focus, because of its potential use against drug-resistant infections. The long-term utilization in agriculture supports the possibility for other applications.

VII. Sources

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4896753/
https://www.ncbi.nlm.nih.gov/pubmed/6624194
http://www.sciencedirect.com/topics/page/Monensin
http://www.scialert.net/fulltext/?doi=ajas.2015.254.265&org=12
https://www.ncbi.nlm.nih.gov/pubmed/20709963
http://ediss.sub.uni-hamburg.de/volltexte/2015/7138/pdf/Dissertation.pdf
http://www.lksh.de/fileadmin/dokumente/Bauernblatt/PDF_Toepper_2013/BB_46_16.11/41_Mahlkow-Nerge.pdf
https://ec.europa.eu/health/documents/community-register/2013/20130128125085/anx_125085_de.pdf
https://www.bayerfarm.de/de/rind/krankheiten/fuetterungskrankheiten/ketose/
http://www.chemspider.com/Chemical-Structure.389937.html (21.05.2017)
http://www.deutschlandfunk.de/doping-fuer-die-turbokuh.697.de.html?dram:article_id=251130 (18.05.2017)
INOUE: Cation Binding by Macrocycles: Complexation of Cationic Species by Crown Ethers. 1. Edition CRC press,. 1990. p.509
NICOLAOU, Kyriacos C. und MONTAGNON, Tamsyn: „Molecules that have changed the world“ Weinheim, Wiley (2008). p.145ff
NICOLAOU, K.C. und SYNDNER, S.A: „ Classics in total synthesis“ Weinheim: Wiley 1996. p.185ff

VIII. Appendix



v

i https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3586448/ (20.06.2017)
ii https://www.farm.com.np/student_articles/ionophores-anti-coccidials-antibiotic-free-poultry-production-antibiotic-resistance-issue/ (20.06.2017)
iii http://www.scialert.net/fulltext/?doi=ajas.2015.254.265&org=12 (20.06.2017)
iv http://www.sciencedirect.com/topics/page/Ionophores (20.06.2017)
v https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2810545/ (20.06.2017)