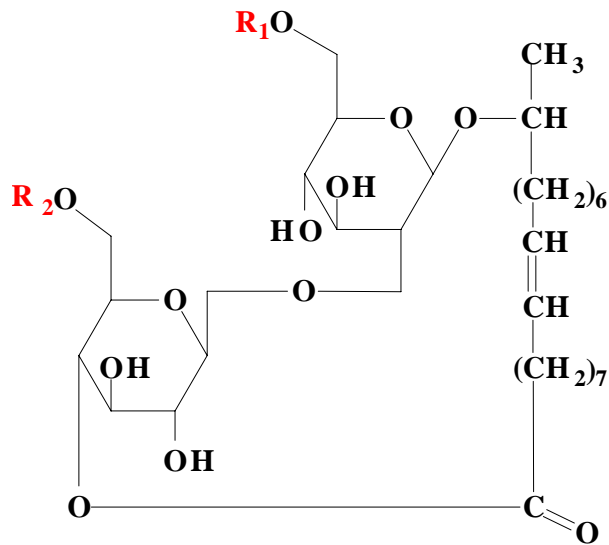
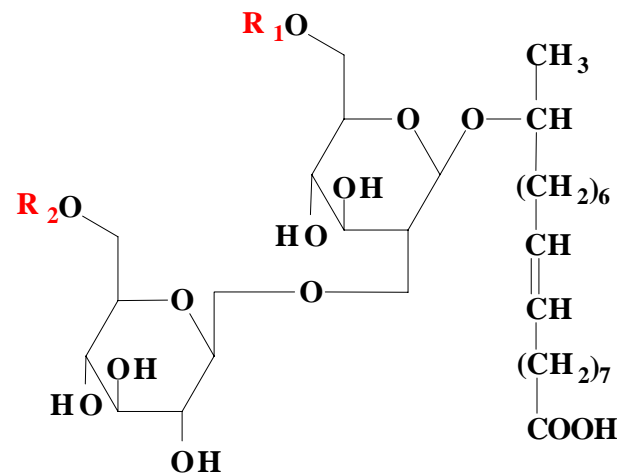


What are Sophorolipids?

Yeast derived glycolipids, consist of **dimeric sophorose** linked to **long-chain hydroxy fatty acids**, produced by *Candida* species (e.g. *C. bombicola* ATCC 22214) when grown on carbohydrates, hydrocarbons, vegetable oils or their mixtures. Produced as a **mixture of six to eight different hydrophobic sophorosides**.

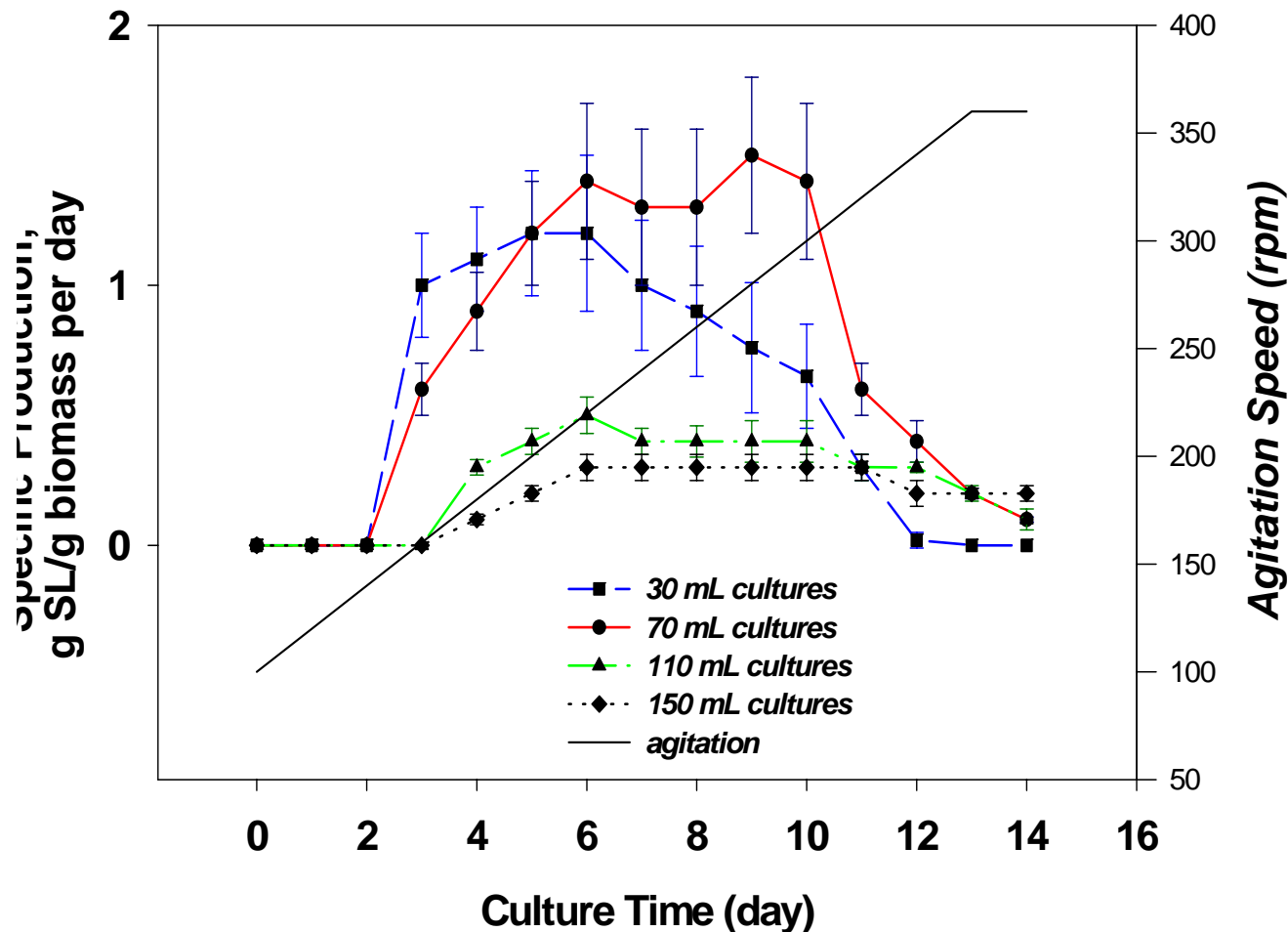


- S₁. R₁ = R₂ = Ac
- S₂. R₁ = H; R₂ = Ac
- S₃. R₁ = Ac; R₂ = H
- S₄. R₁ = R₂ = H



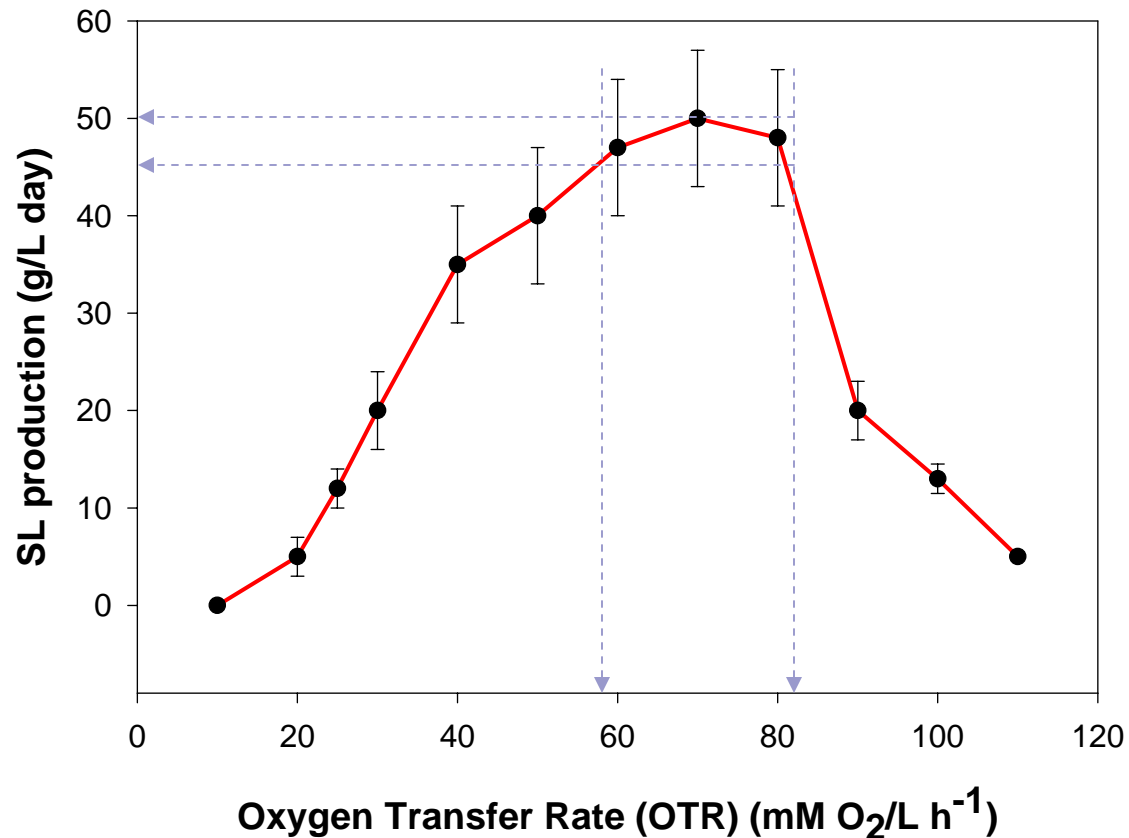
- S₅. R₁ = R₂ = Ac
- S₆. R₁ = H; R₂ = Ac
- S₇. R₁ = Ac; R₂ = H
- S₈. R₁ = R₂ = H

SL production with linear increase in agitation at different volumetric levels - Existence of an optimal aeration window



An oxygen transfer rate (OTR) between 50 and 80 mM O₂/L h⁻¹ gave the maximum volumetric product formation (1-1.5 g/L h⁻¹) and SL volumetric yield (350 g/L)

**SL production depends on the aeration conditions.
There exists an optimal range of OTR in flasks.**



V Guilmanov, A Ballistreri, G Impallomeni, R.A. Gross, "Oxygen Transfer Rate and Sophorose Lipid Production by *Candida bombicola*", *Biotechnol. and Bioeng.* 77(5), 489-494 (2002)

Simple Derivatives of Natural Products

Sophorolipid mixture

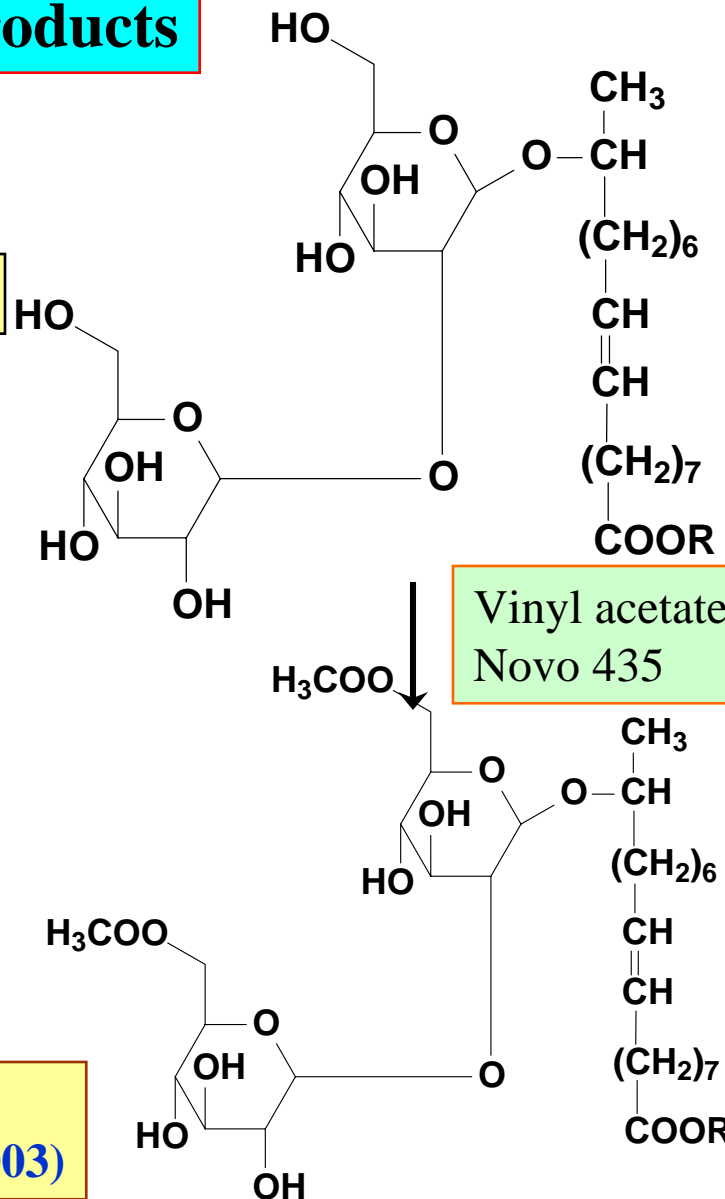
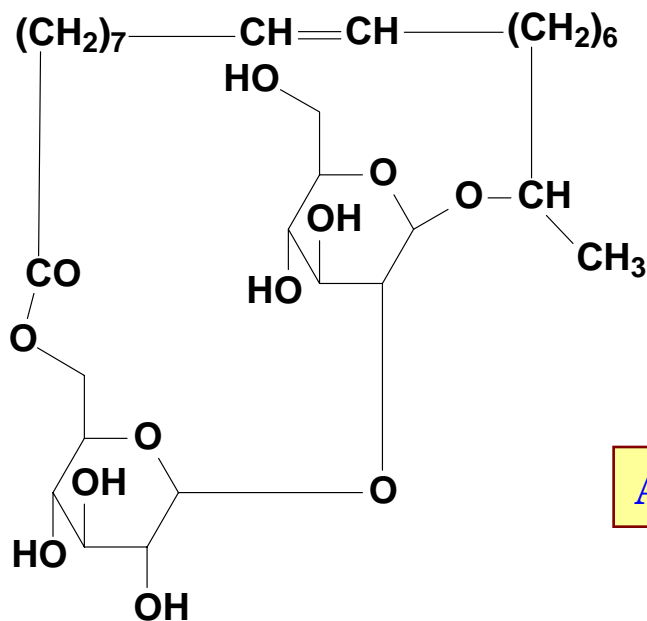
NaOR

R = Me, Et, Bu and Hex

Novo 435

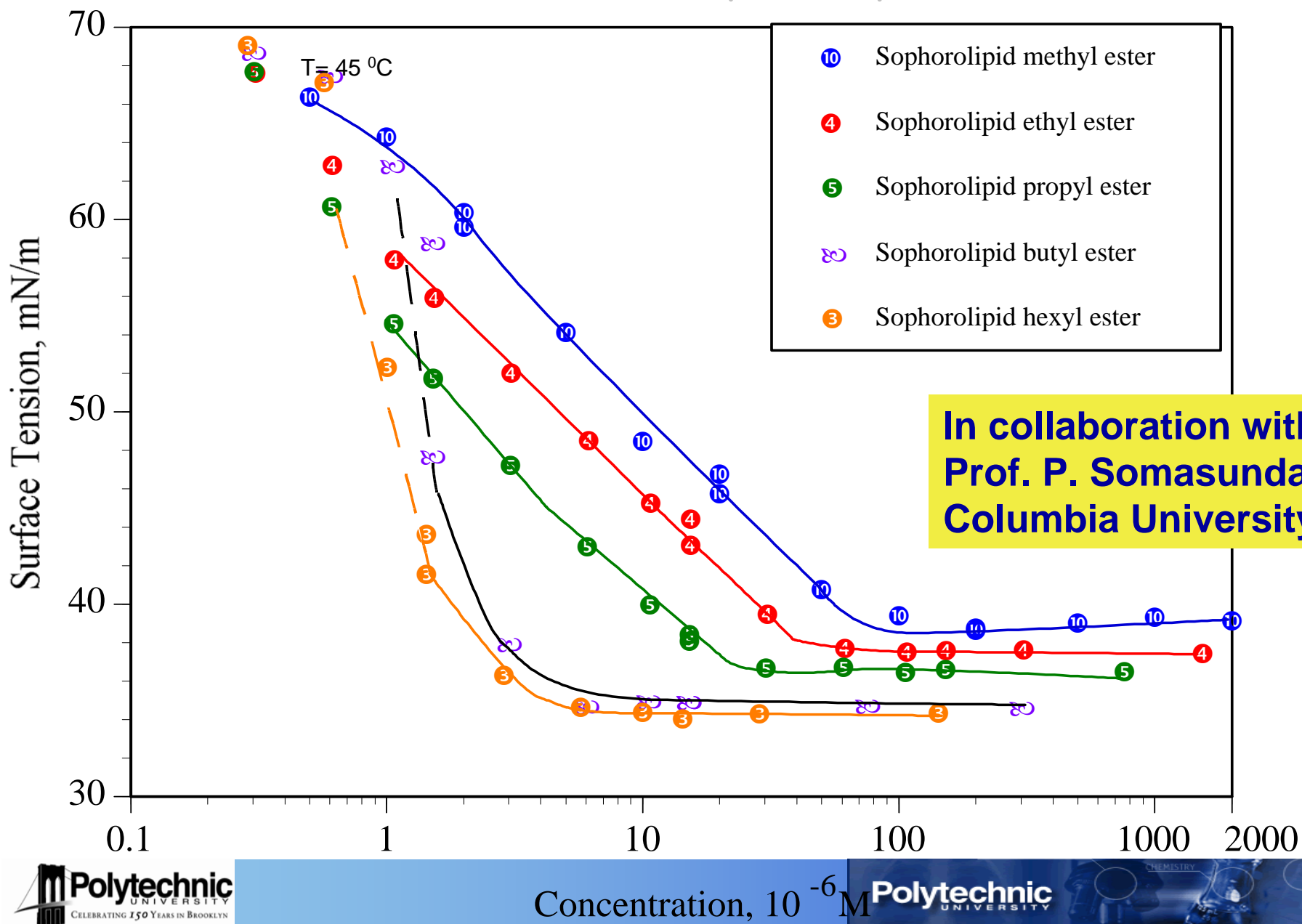
Vinyl acetate, Novo 435

Also Monoacylation

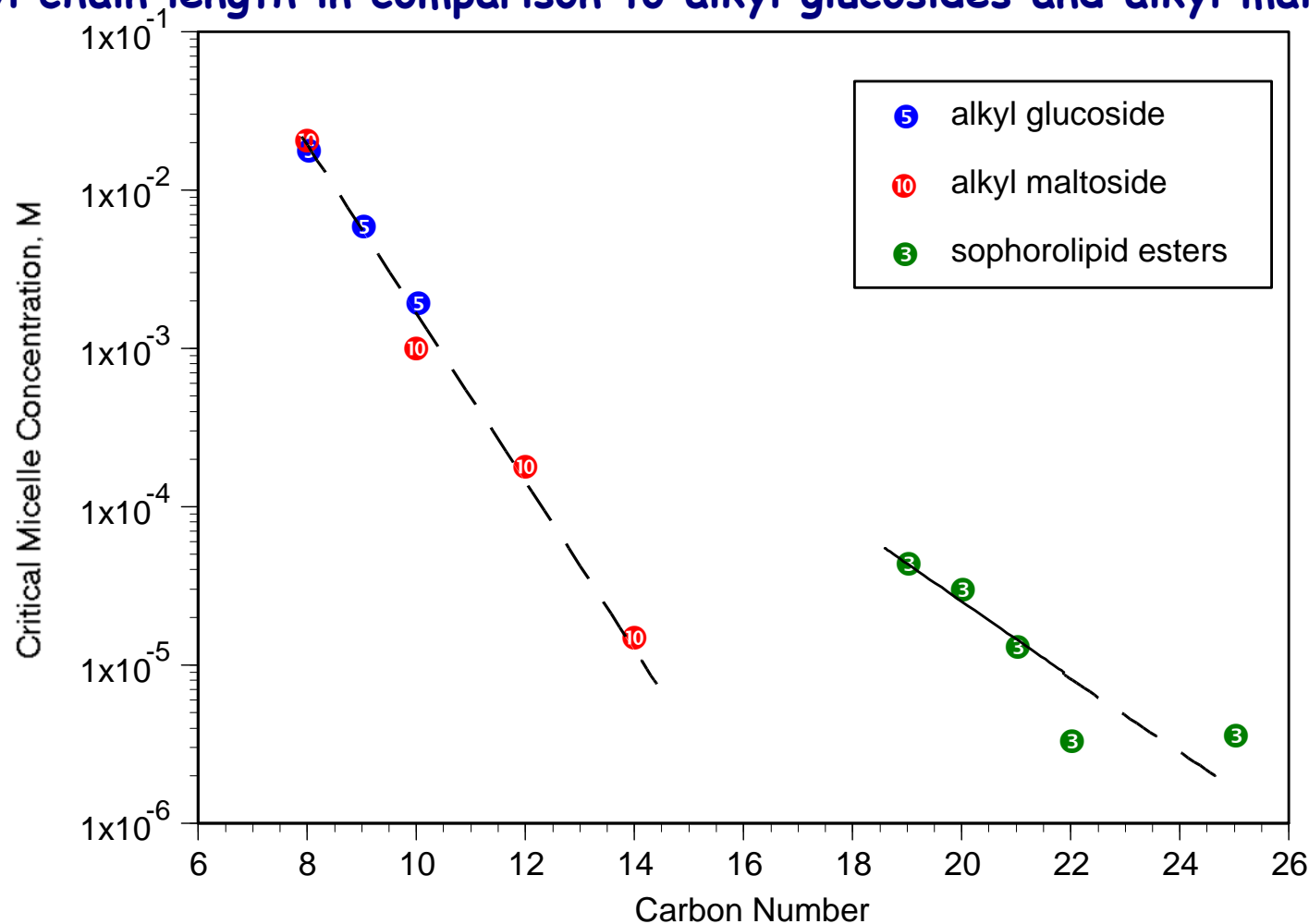


Bisht, Gross., *J. Org. Chem.*, 1999, 64, 780
 Singh, S. K, Gross...*J. Org. Chem.*; 68(14); 5466 (2003)

Surface Tension of Esters of Sophorolipid

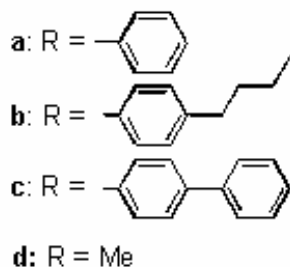
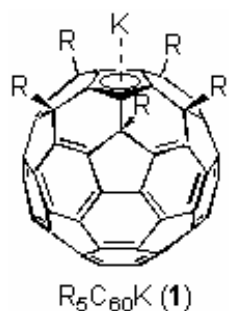


Critical micelle concentration of sophorolipid esters as a function of alkyl chain length in comparison to alkyl glucosides and alkyl maltosides

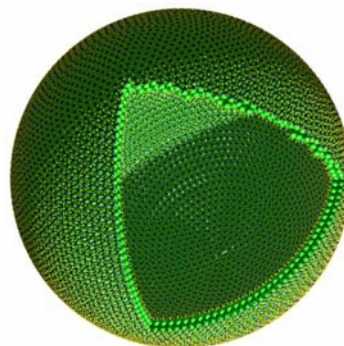


Zhang, L., Somasundaran, P., Singh, S. K., Felse, A. P., Gross, R.A. Synthesis and interfacial properties of sophorolipid derivatives *Colloids and Surfaces A: Physico-chem. Eng. Aspects* 240 (2004) 75-82

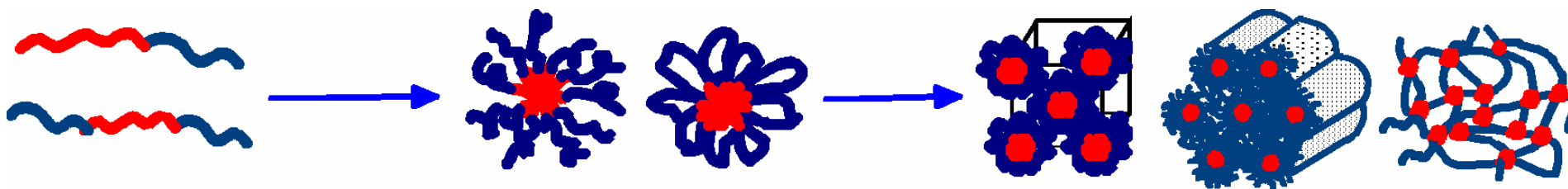
Self-assembly of surfactants and block copolymers



In water



Science, 2001,
291, 1944-1947

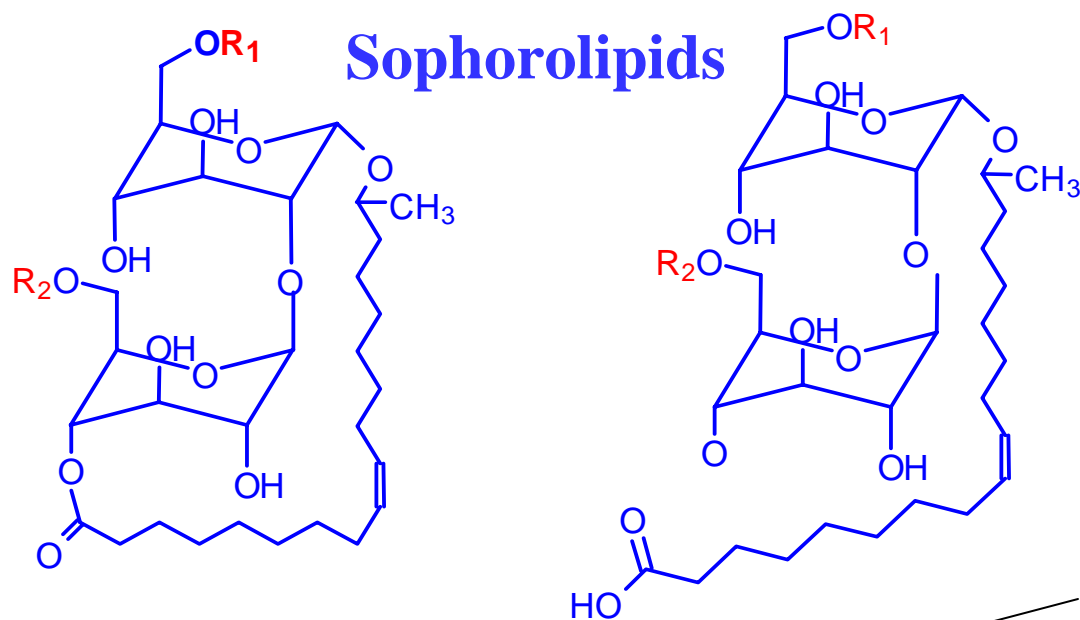


Macromolecules, 1998, 31, 5300-5308; 7746-7755; 1999, 32, 5836-5845;
6326-6332. *J. Polym. Sci., Polym. Phys.*, 1998, 36, 889-900.

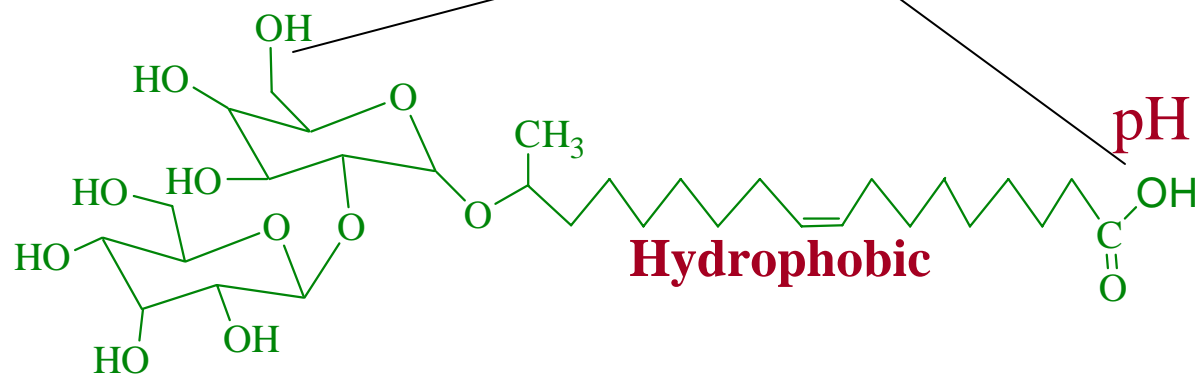
Self-Assembling of Sophorolipids

In collaboration with:

Prof. Shuiqin Zhou --CUNY College of Staten Island



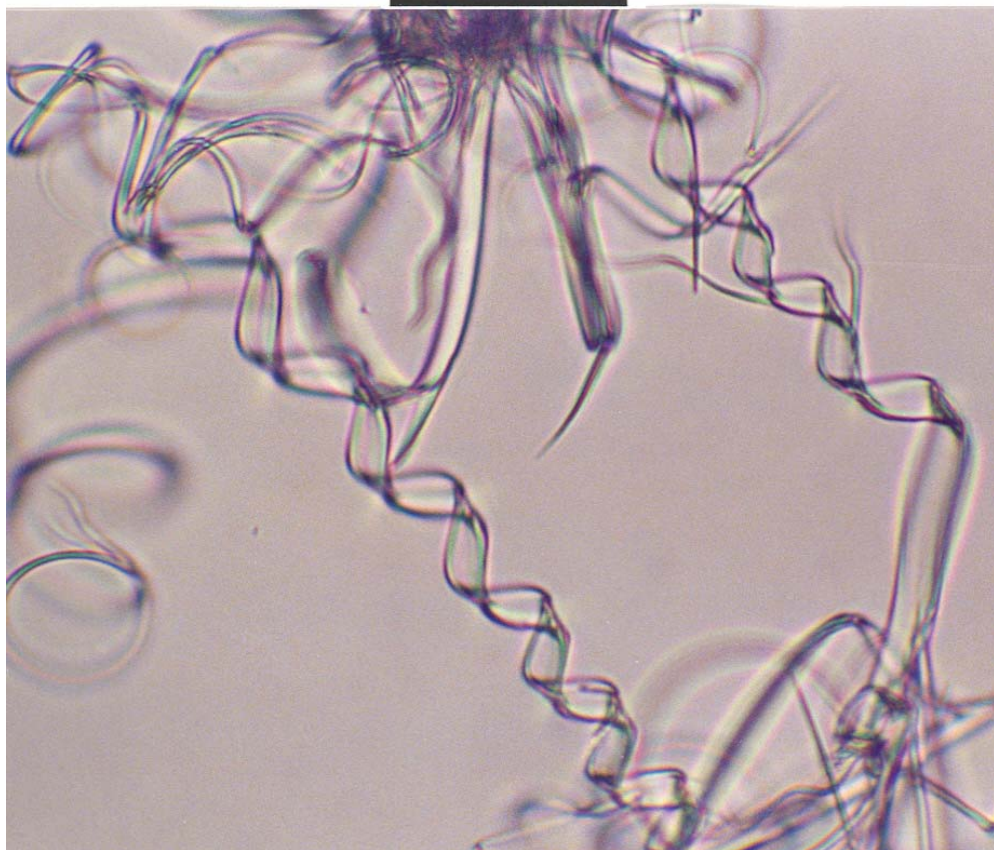
Free acidic SL



Self-Assembling of Sophorolipids

Free acidic SL dissolved in dilute HCl, final pH = 4.1, C = 2.2 mg/mL

50 μm



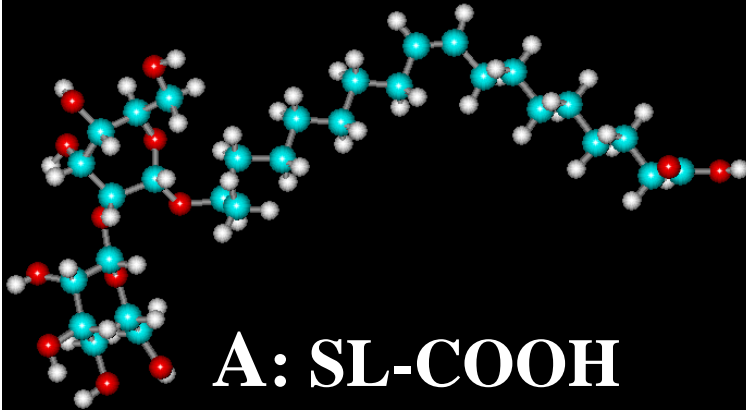
50 μm



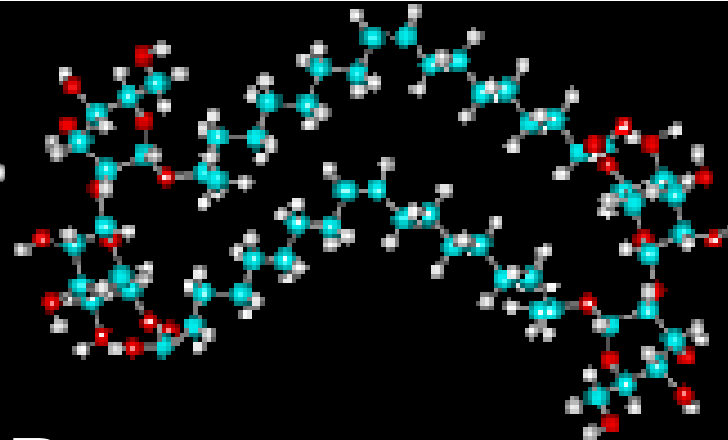
Big helical ribbons (e.g., $\sim 11 \mu\text{m}$ wide and hundreds μm long)

Zhou, S., Xu, Chang., Wang, J., Gao, W., Akhverdiyeva., Shah, V., Gross, R. A.

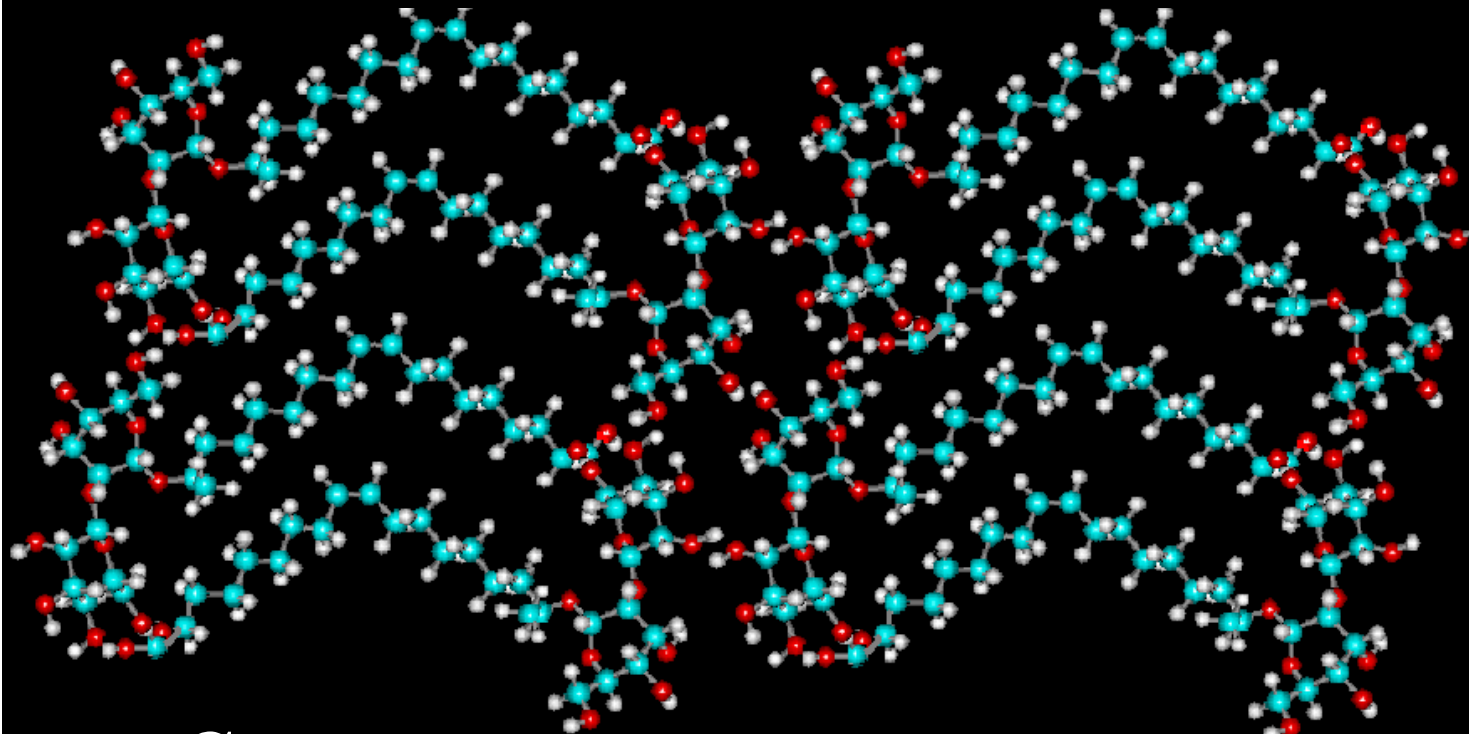
Supramolecular Assemblies of a Naturally Derived Sopholipid. *Langmuir* 2004, 20, 7926-7932



A: SL-COOH



B: interdigitated dimer



C: Long-range interdigitated packing

Molecular modeling of SL-COOH molecules (A) and a possible interdigitated lamellar packing model of SL-COOH molecules in the giant ribbons (C).

Reported Effects of Sophorolipids

- ❑ **Seemingly well tolerated in mice and rats without conflicting reports.**
- ❑ **No toxicological effect of non-modified or native sophorolipids in rats with 208 mg/kg/day**
- ❑ **oral LD 50 (rates + mice): 10-16 g/kg; mice: 5.8 to 6.6 g/kg. The oral LD of SL is estimated at 12.5 g/kg.**

Ikeda Y. et al. J. of Toxicological Sciences, 11 (3): 197-211 and 213-224, August 1986.

- ❑ **No measurable consequence with 12.5 g/kg SL in rates or 6 g/kg in mice.**

Otto R. T. et al. Appl. Microbiol. Biotechnol (1999) 52: 495-501.

Previous work done by our group

Sophorolipid mixture

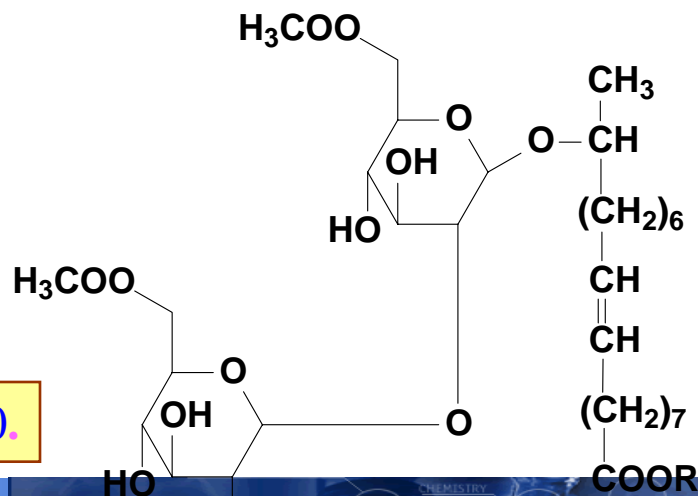
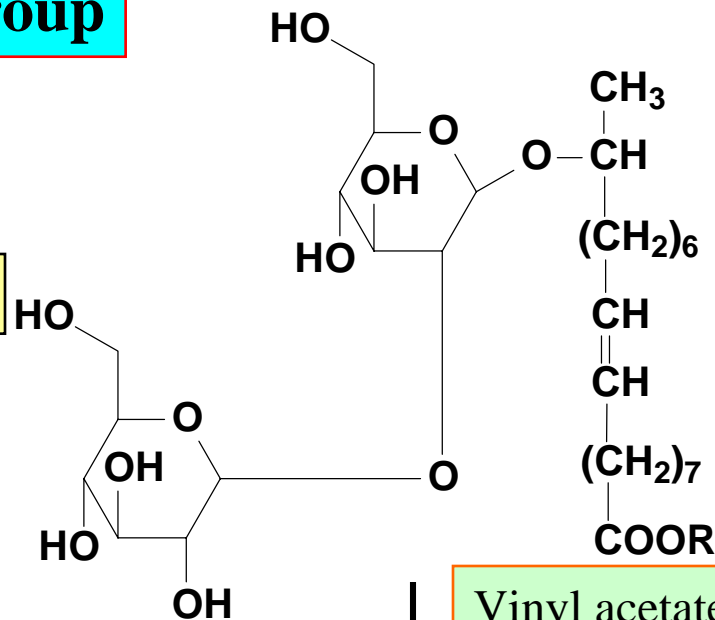
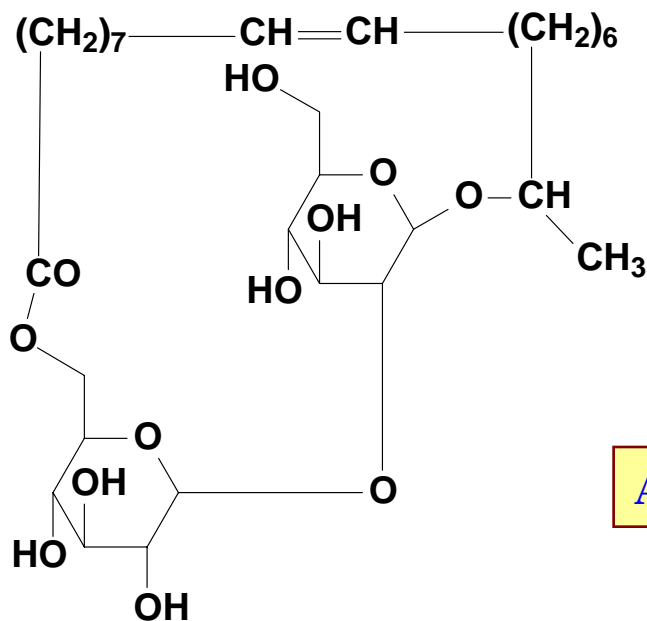
NaOR

R = Me, Et, Bu and Hex

Novo 435

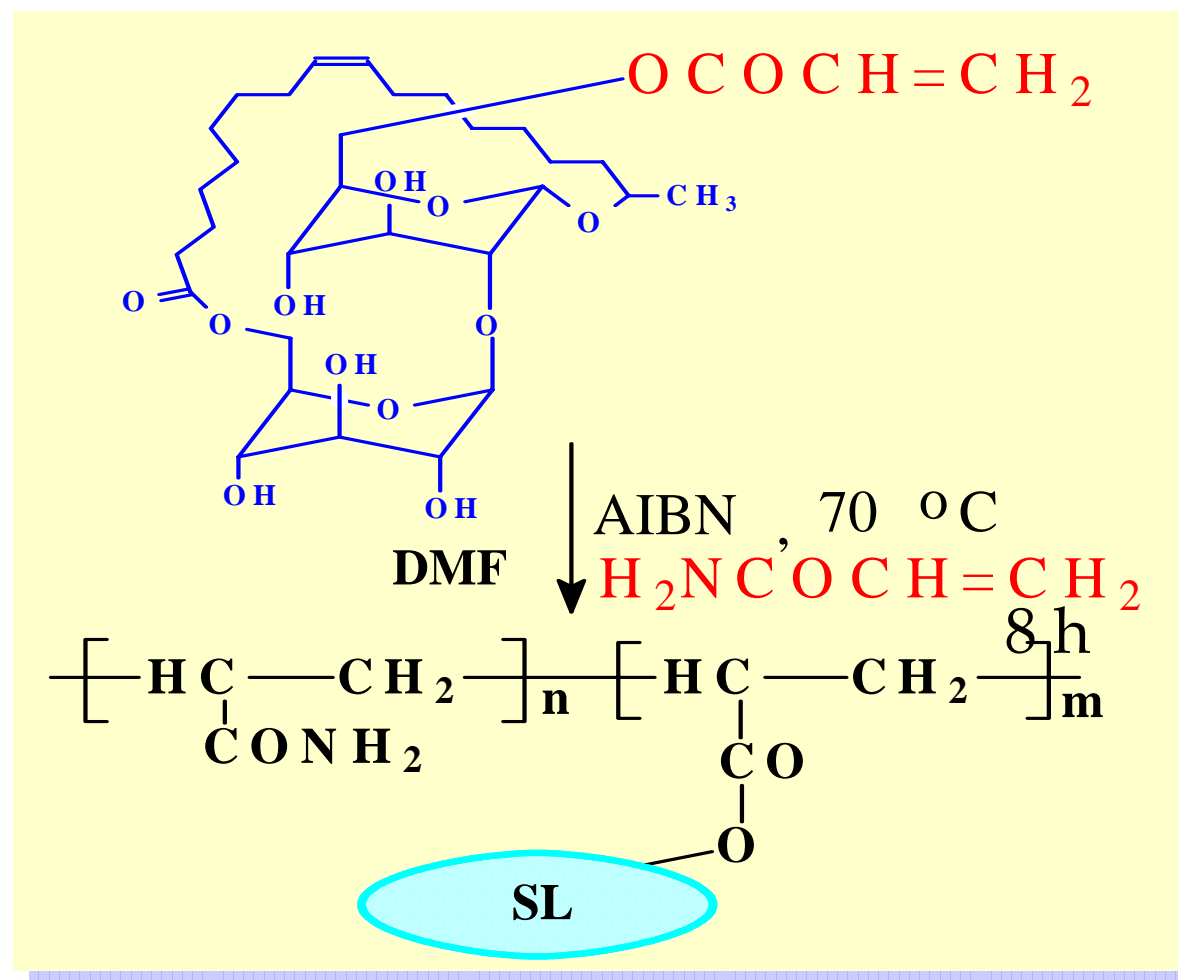
Vinyl acetate, Novo 435

Also Monoacylation



Bisht, Gross and Kaplan, *J. Org. Chem.*, 1999, 64, 780.

Copolymerization of Monoacryl Sophorolactone with Acrylamide



Bisht, Gao, Gross, *Macromolecules*, (2000).

Antimicrobial Activity

Microorganisms	Hexyl SL (mg/ml)	SL-Acid (mg/ml)	SL Monoacetate Ethyl Ester (mg/ml)	SL DiAcetate Ethyl Ester (mg/ml)	SL Methyl Ester (mg/ml)	Natural SL (mg/ml)
<i>Escherichia coli</i>	1.67	5.00	5.00	5.00	1.67	5.00
<i>Moraxella sp.</i>	1.67	5.00	0.02	0.06	0.06	5.00
<i>Ralstonia eurtropa</i>	5.00	5.0	5.00	5.00	5.00	5.00
<i>Rhodococcus erythropolis</i>	> 5.00	0.56	0.007	5.00	5.00	5.00
<i>Salmonella choleraesuis</i>	5.00	5.00	5.00	5.00	1.67	5.00
<i>Bacillus subtilis</i>	1.67	N/A	0.06	0.06	0.56	5.00
<i>Streptococcus agalatae</i>	1.67	5.00	0.007	0.02	0.06	5.00
<i>Candida albicans</i>	1.67	5.00	5.00	5.00	1.67	5.00
<i>Candida antartica</i>	1.67	5.00	0.02	0.06	0.06	5.00
<i>Candida tropicalis</i>	1.67	5.00	0.007	0.02	0.06	5.00

Shah V, Doncel GF, Seyoum T, Eaton KM, Zalenskaya I, Hagver R, Azim A, Gross R. Sphorolipids, microbial glycolipids with anti-human immunodeficiency virus and sperm-immobilizing activities. *Antimicrob Agents Chemother* 49:1-8 (2005)

Bioactive Agents

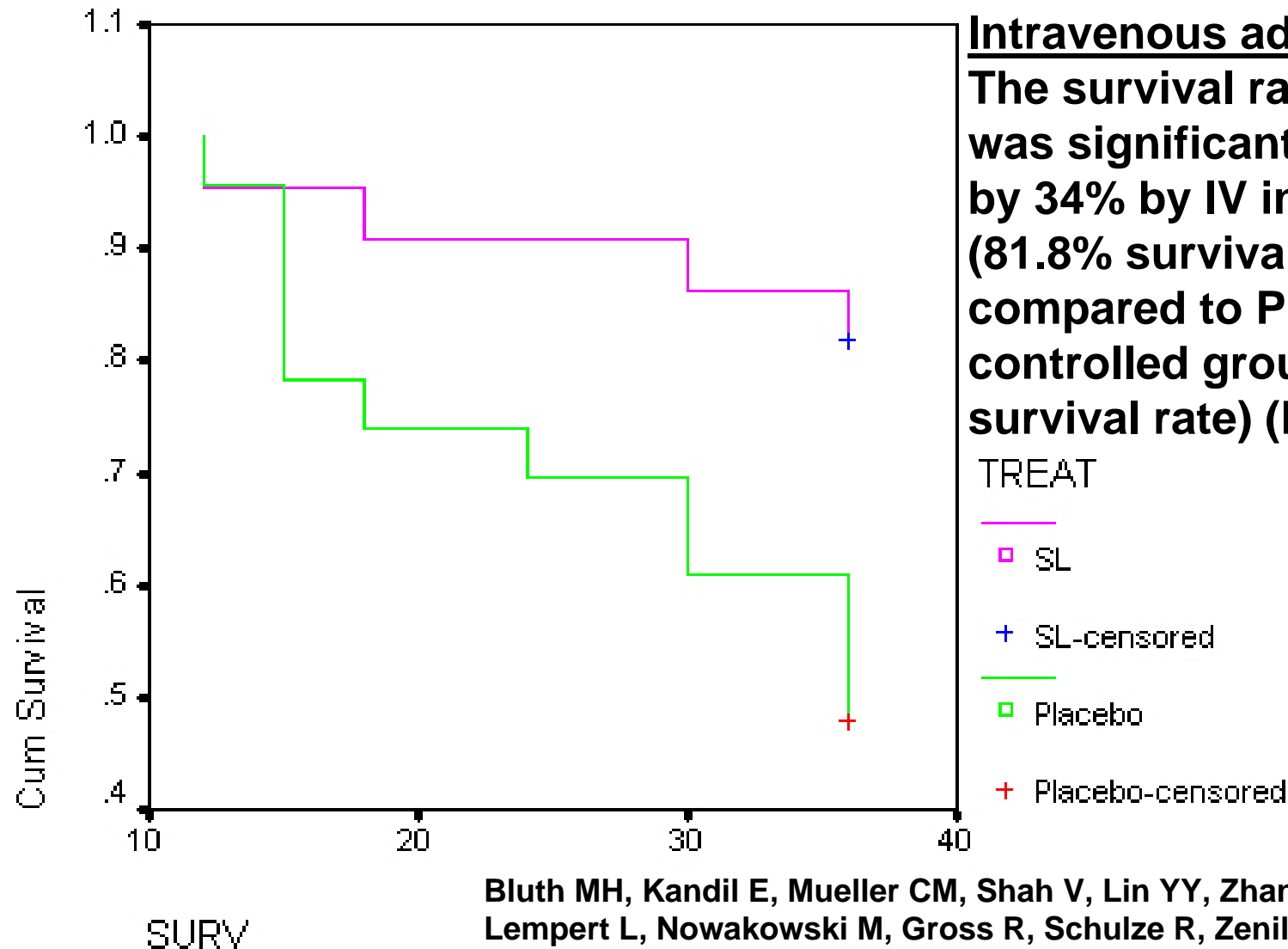
- Septic shock antagonists

To test the benefits of Sophrolipids in an in vivo system model of sepsis, 100 Male Adult Sprague-Dawley rats (200-240g) were used.

Cecal Ligation to induce sepsis

- All animals were anesthetized with an intraperitoneal injection of Nembutal (40mg/kg).
- The cecum was ligated just below the ileocecal valve with a 3-0 silk ligature. The antimesenteric cecal surface was punctured with a 16-gauge needle proximal to the ligature. The abdominal incision was then closed in two layers.
- The animals were randomly assigned to one of two groups (n=25 per group).
- To determine the efficacy of intravenous administration of Sophrolipids, animals were randomly assigned to two additional groups (n=25 per group).
- Control animals received physiological saline, while experimental animals received sophrolipids (5 mg/kg) intravenously (IV) via tail vein injection at the end of the operation.
- The animals were then monitored over 36 hours and survival rate was compared between the experimental and control groups. The endpoint of the study was lethality

Survival Functions



Bluth MH, Kandil E, Mueller CM, Shah V, Lin YY, Zhang H, Dresner L, Lempert L, Nowakowski M, Gross R, Schulze R, Zenilman ME. Sophorolipids block lethal effects of septic shock in rats using a cecal ligation and puncture model of experimental sepsis. *Crit Care Med* Vol. 34, No. 1 (2006).