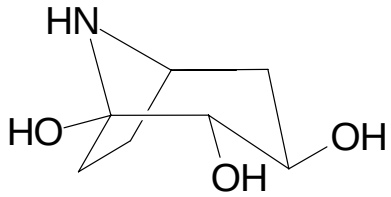
# Natural Products with High Chromatographic Challenges - Ceramides and Calystegines

Annett Opitz°, Yvonne Sichhart*, Reinhard Neubert°, Birgit Dräger*

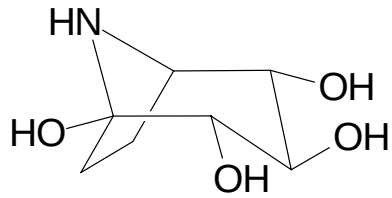
Martin Luther University Halle-Wittenberg
° Biopharmacy at the Institute of Pharmacy
*Pharmaceutical Biology at the Institute of Pharmacy

# The Challenges

## Natural products



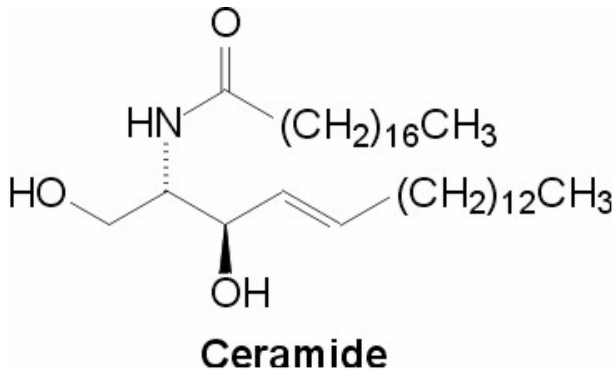

**Calystegine $A_3$**




**Calystegine $B_2$**

## Calystegines

- Hydrophilic alkaloids of many Solanaceae, e.g. potato, tomato
- Strong glycosidase inhibitors



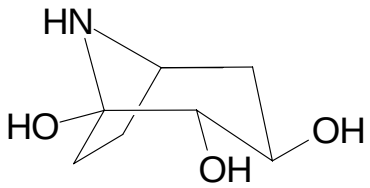

**Ceramide**

## Ceramides

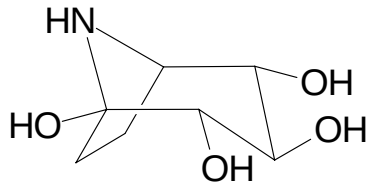
- Essential components of biological membranes
- Inducers of apoptosis

# The Challenges

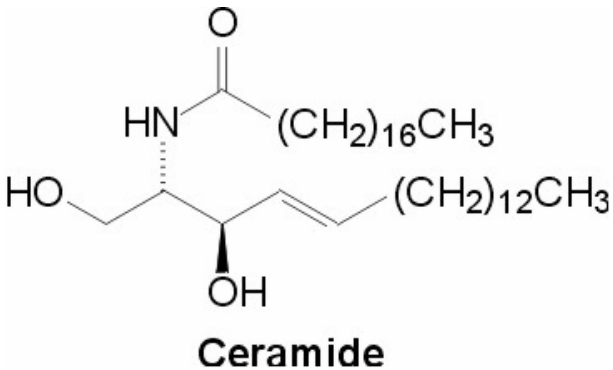
## Natural products




**Calystegine $A_3$**




**Calystegine $B_2$**

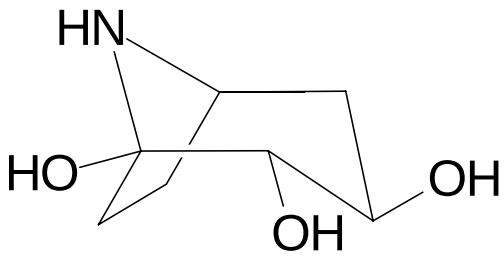



**Ceramide**

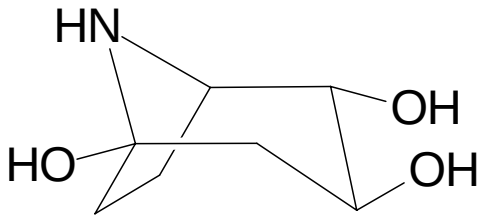
## ... and their matrix

# Calystegines

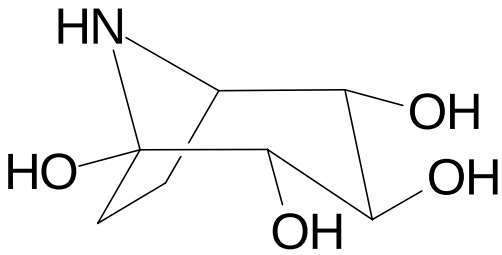
- Hydrophilic alkaloids
- UV absorption very weak
- Low concentrations in plant tissues



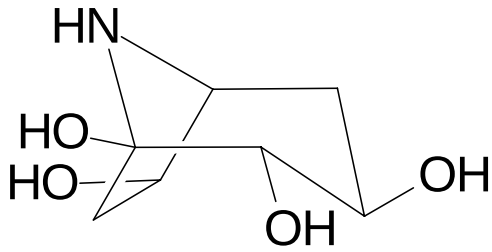

**Calystegine $A_3$**




**Calystegine $A_5$**




**Calystegine $B_2$**



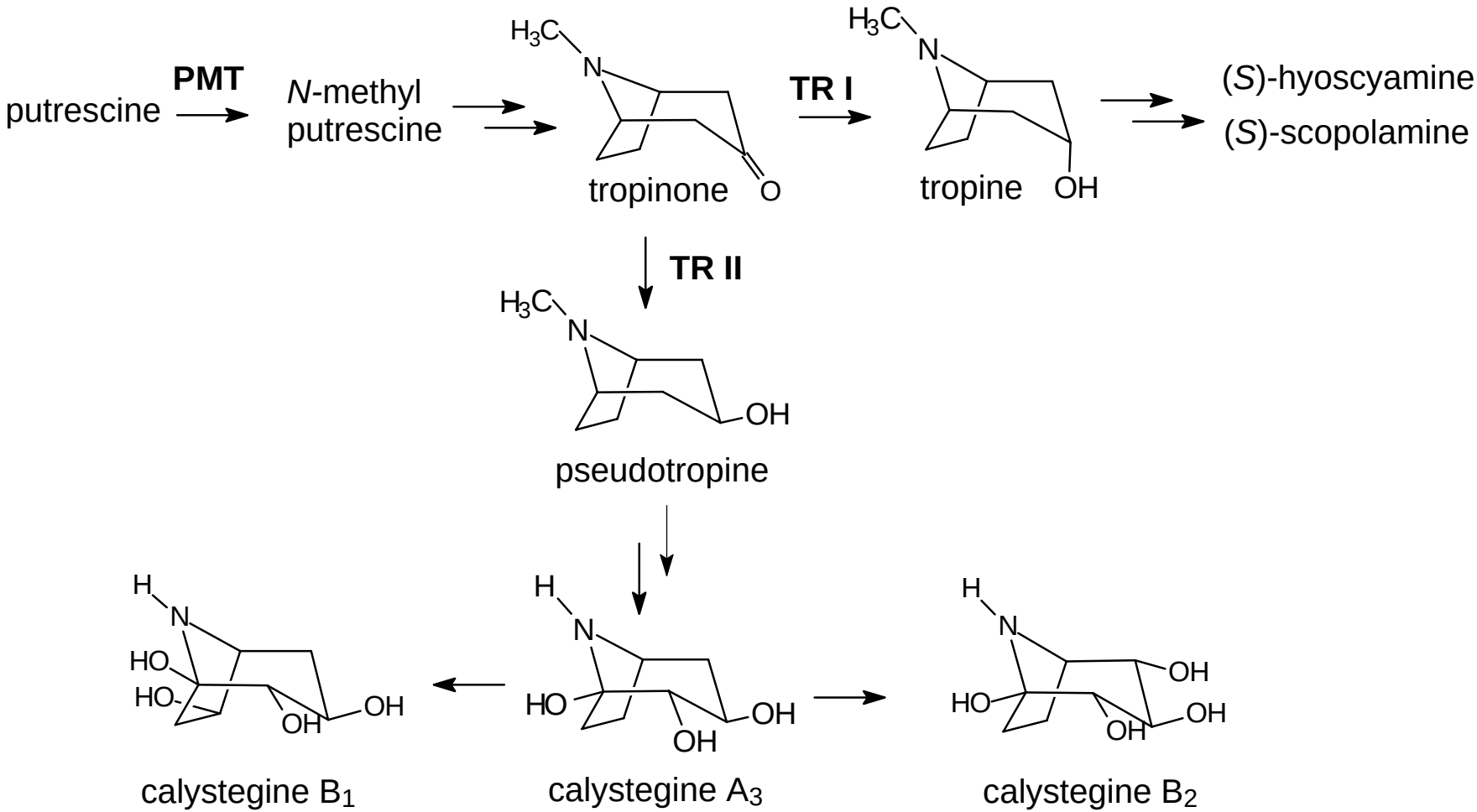

**Calystegine $B_1$**

*Atropa belladonna*
Deadly nightshade
Contains atropine and calystegines

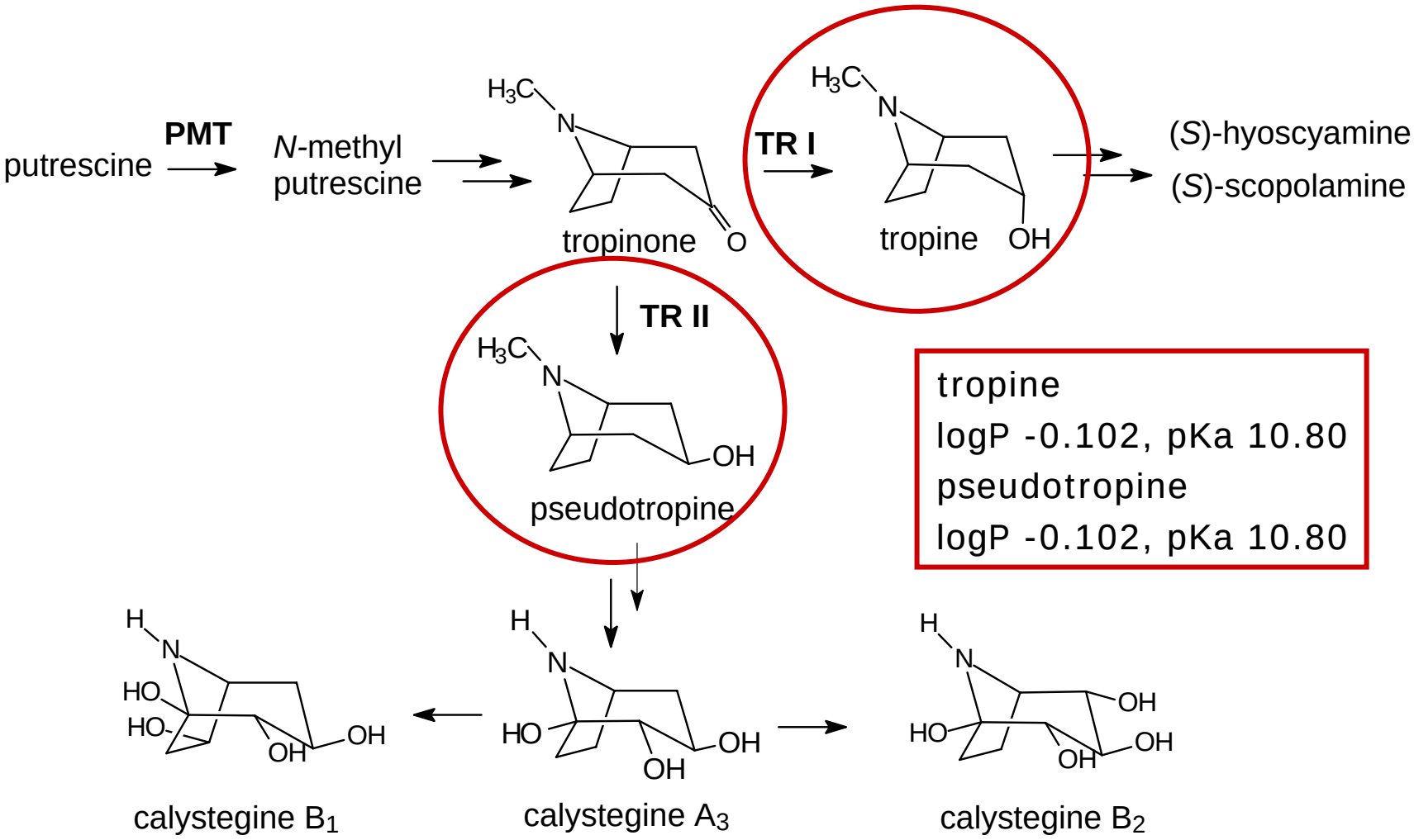
*Lycopersicum esculentum*
Tomato
Contains calystegines

*Solanum tuberosum*
Potato
Contains calystegines

# Biosynthetic origin of calystegines




**PMT** = putrescine *N*-methyl transferase
**TRI** = tropine forming tropinone reductase
**TRII** = pseudotropine forming tropinone reductase




**PMT** = putrescine *N*-methyl transferase
**TRI** = tropine forming tropinone reductase
**TRII** = pseudotropine forming tropinone reductase

# Alkaloid isomers are separated

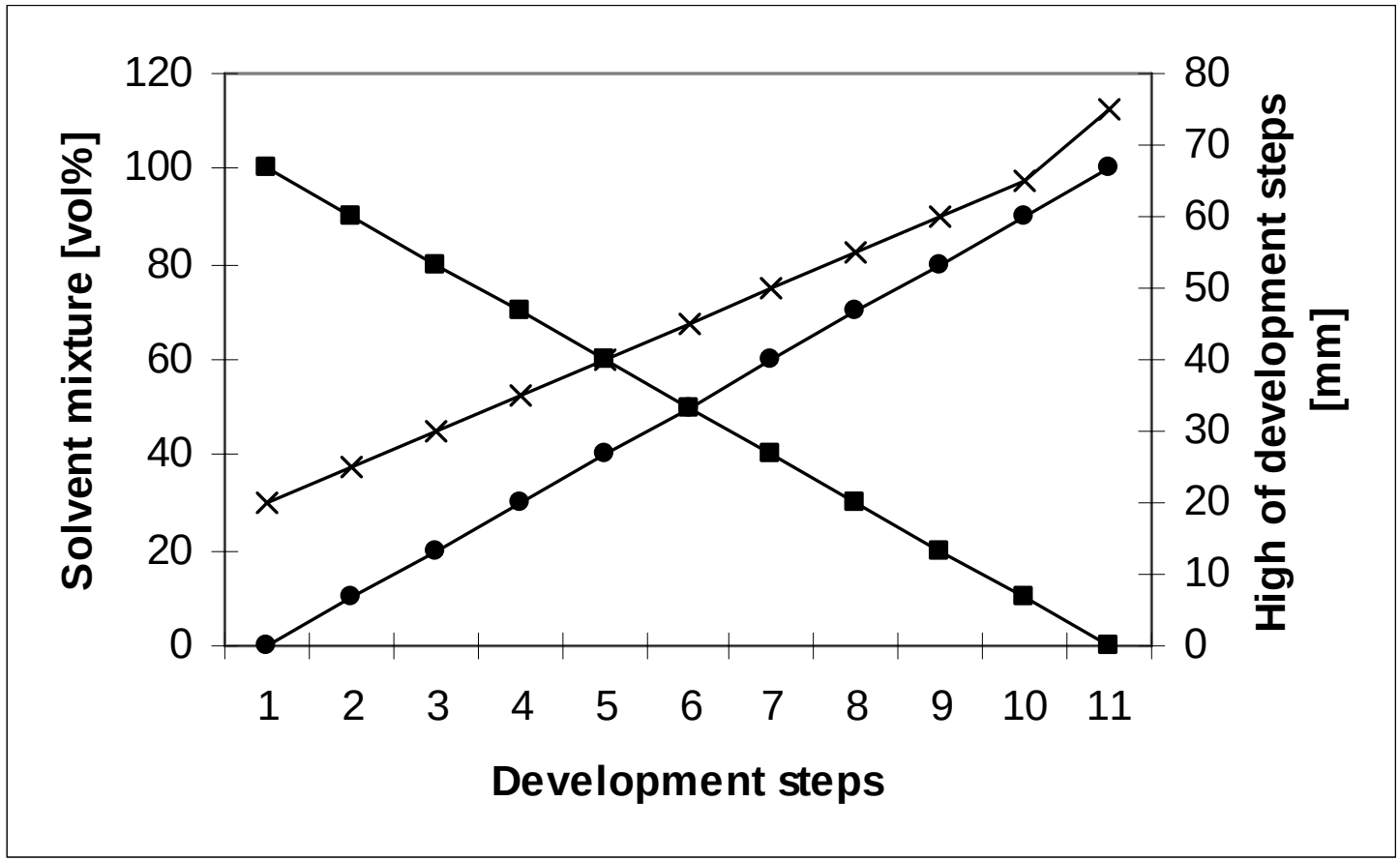

tropinone

pseudotropine

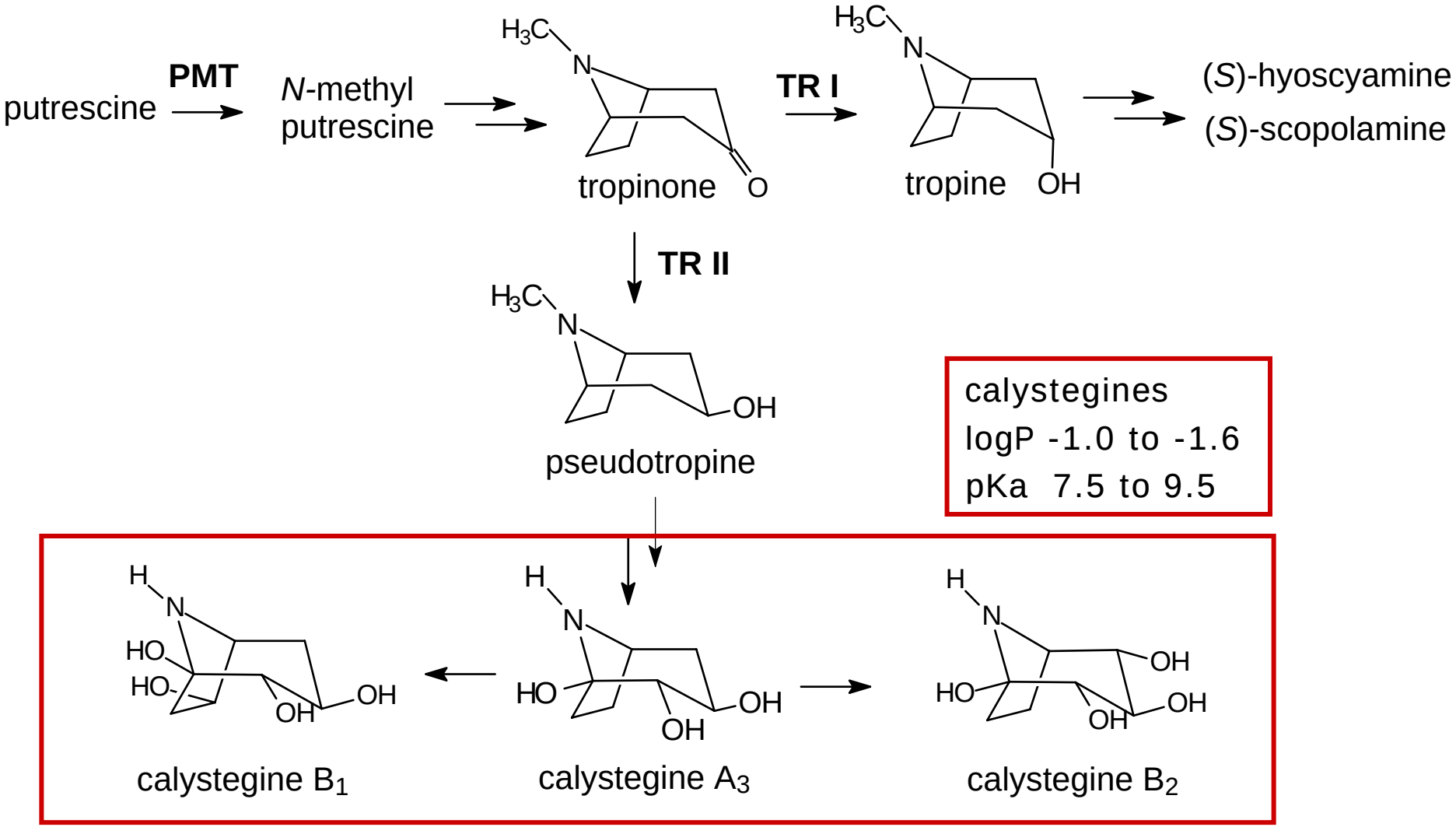
tropine

Detection: Dragendorff reagent, var. Munier
Bismuth nitrate, tartaric acid, potassium iodide

# AMD separation




HPTLC plates: Silica gel
Elution gradient profile: ▭ methanol; 🕮 chloroform; x eluent front




**PMT** = putrescine *N*-methyl transferase
**TRI** = tropine forming tropinone reductase
**TRII** = pseudotropine forming tropinone reductase

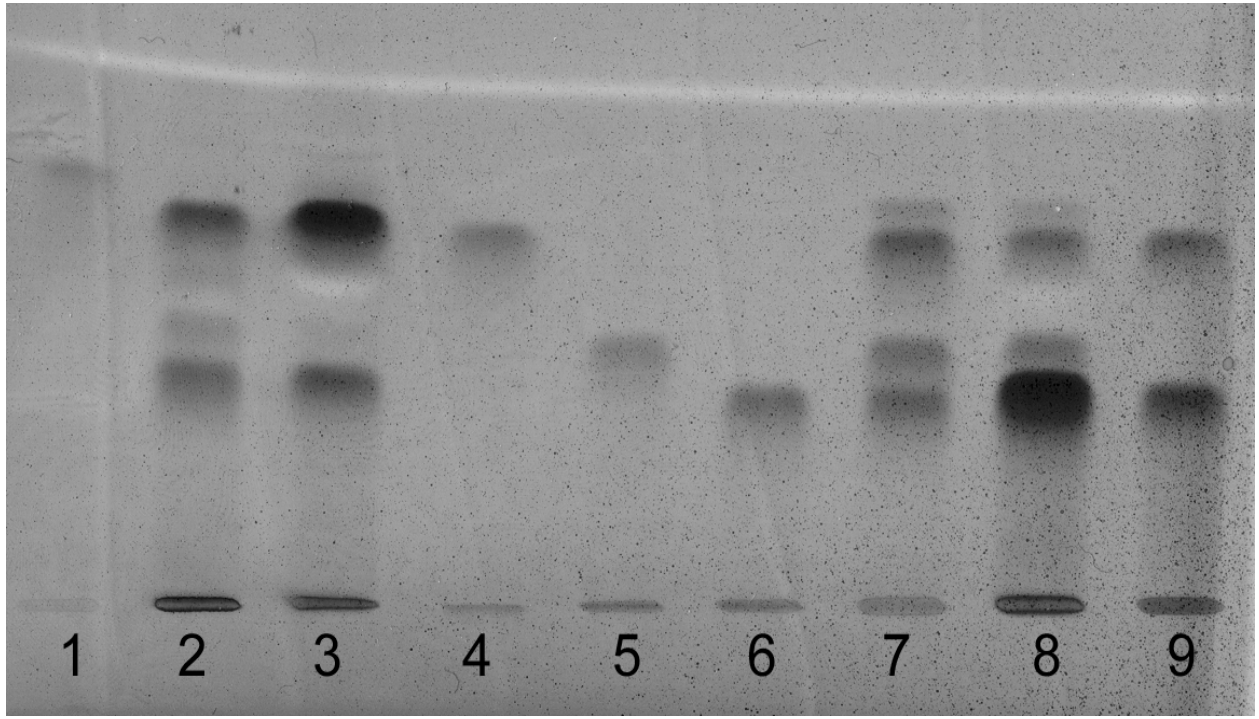
# Calystegine isomers are separated

2 *Calystegia sepium*

3 *Atropa belladonna*

8 *Hyoscyamus muticus*

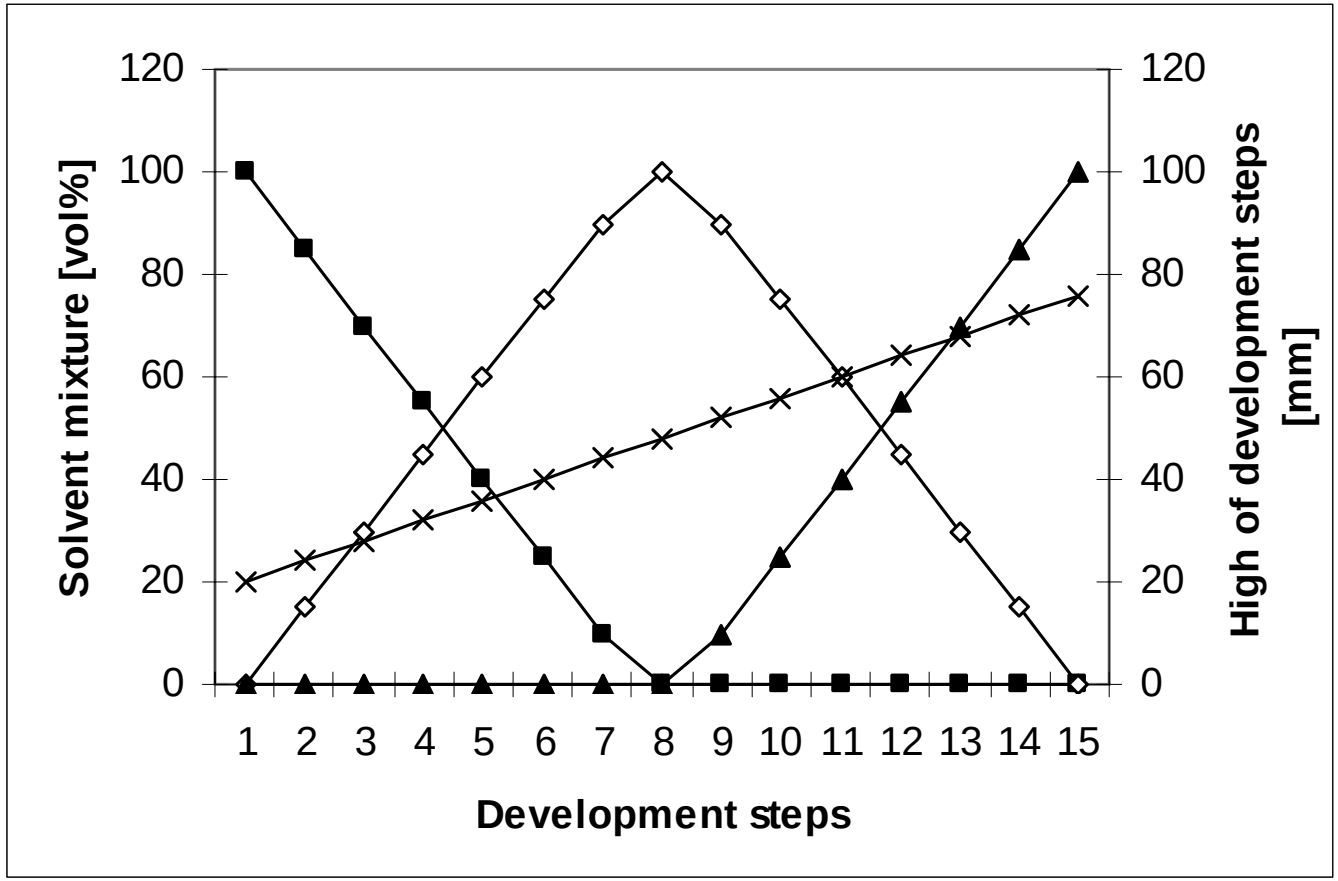
9 *Solanum tuberosum* potato




standards $A_5$ $A_3$ $B_1$ $B_2$ all

Detection: 1. silver nitrate in acetone
2. sodium hydroxide in ethanol

# AMD separation




HPTLC plates: Silica gel
Elution gradient profile: ■ methanol; ▲ propanol
◇ ethanol; x eluent front

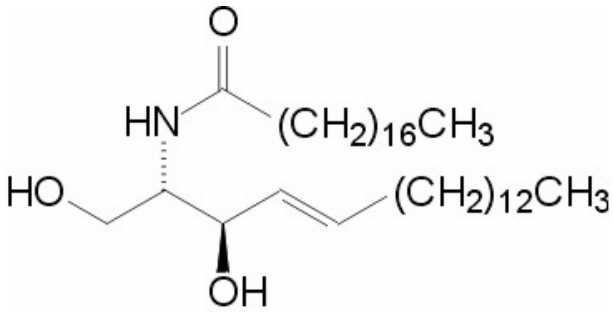
# Characteristics and advantages of AMD separation

Compounds are separated that posess

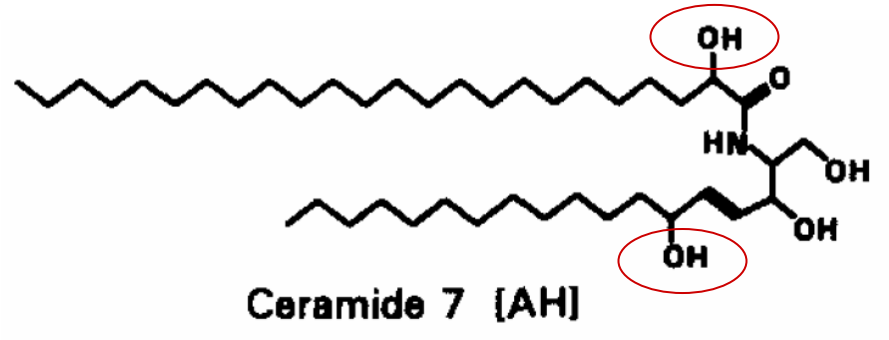
- high polarity
- high structural similarity
- no UV absorption

Scholl, Asano, Dräger (2001)
Automated multiple development thin layer chromatography for 13 calystegines and their biosynthetic precursors
J. Chromatography A 928, 217-224

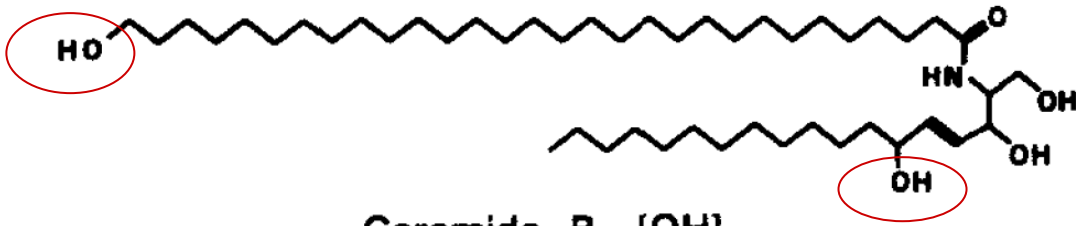
# Ceramides




Ceramide



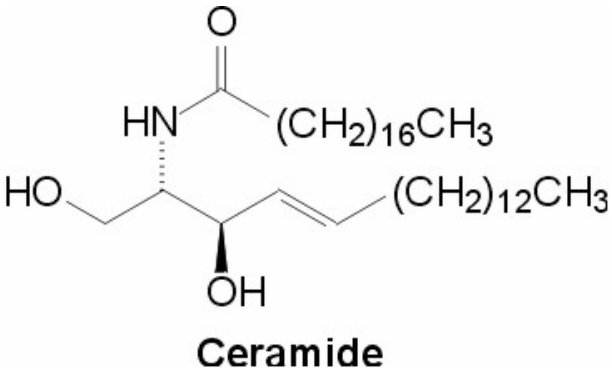

Ceramide 7 [AH]



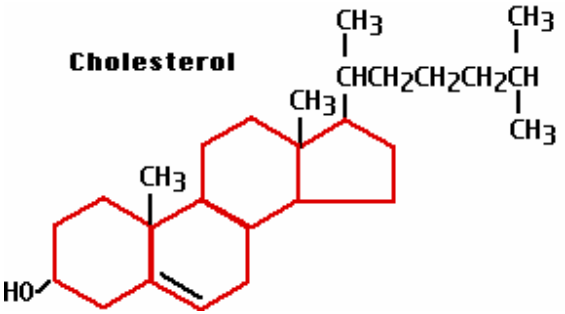

Ceramide B [OH]

- Essential components of biological membranes
- broad variety of structures

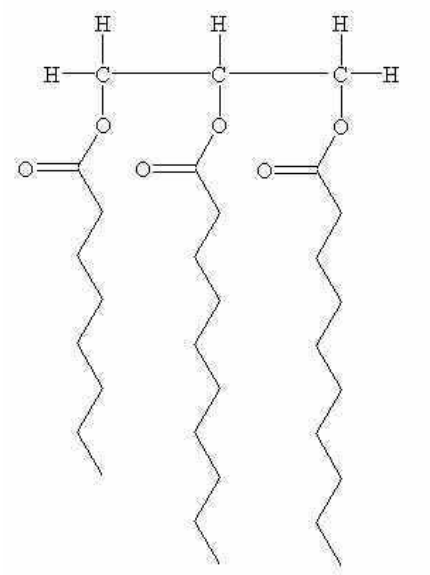
# Skin lipids




**Ceramide**

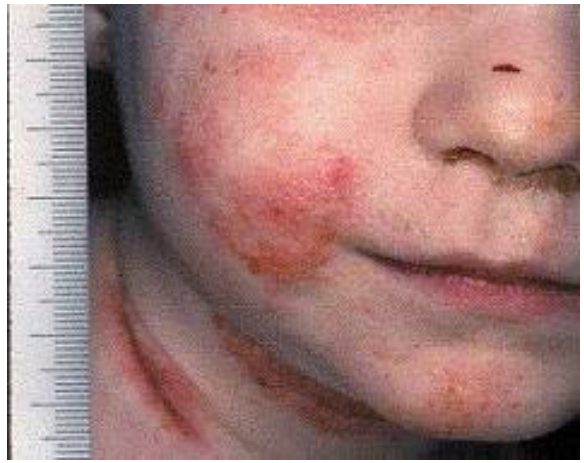



and other sterols

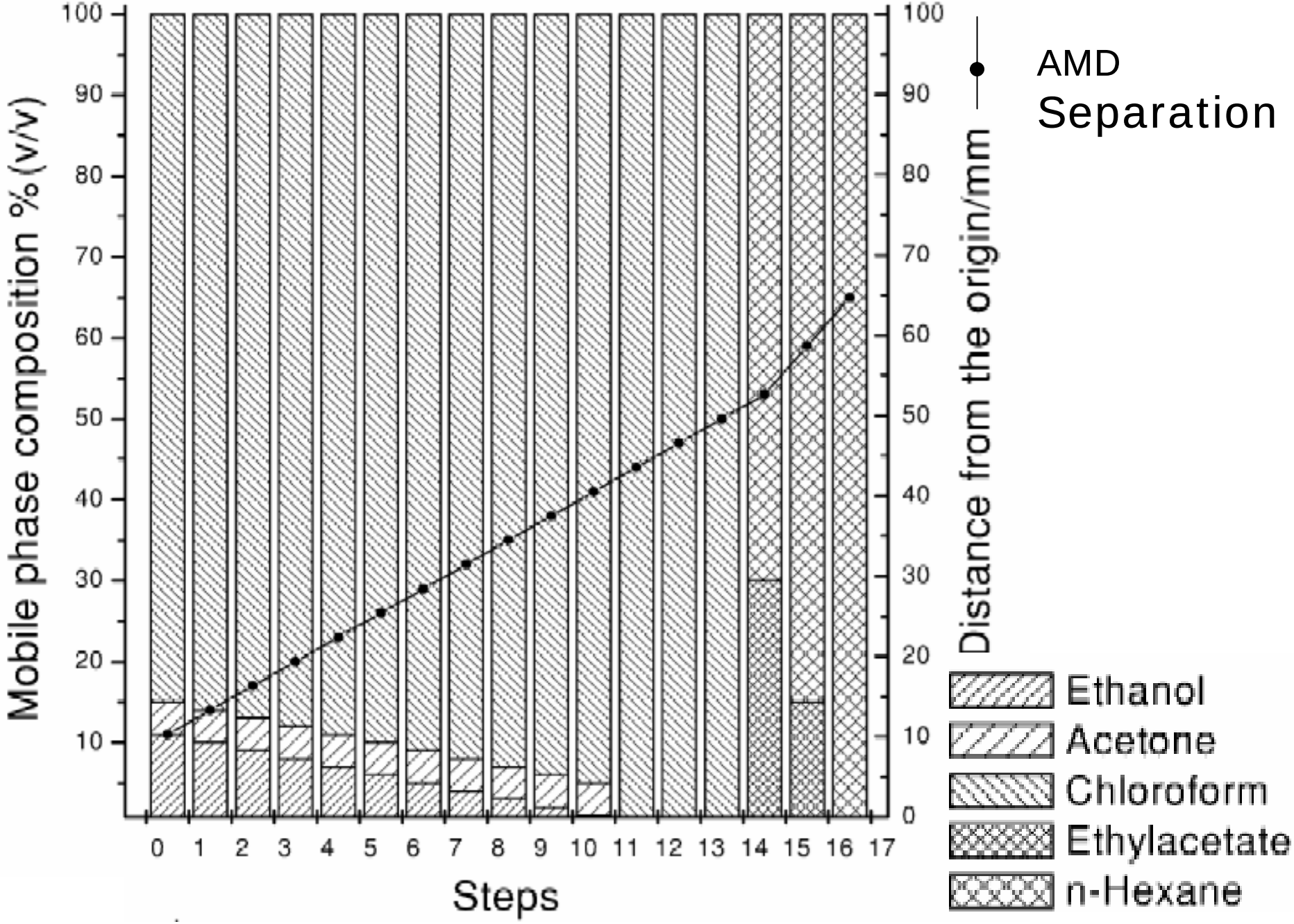


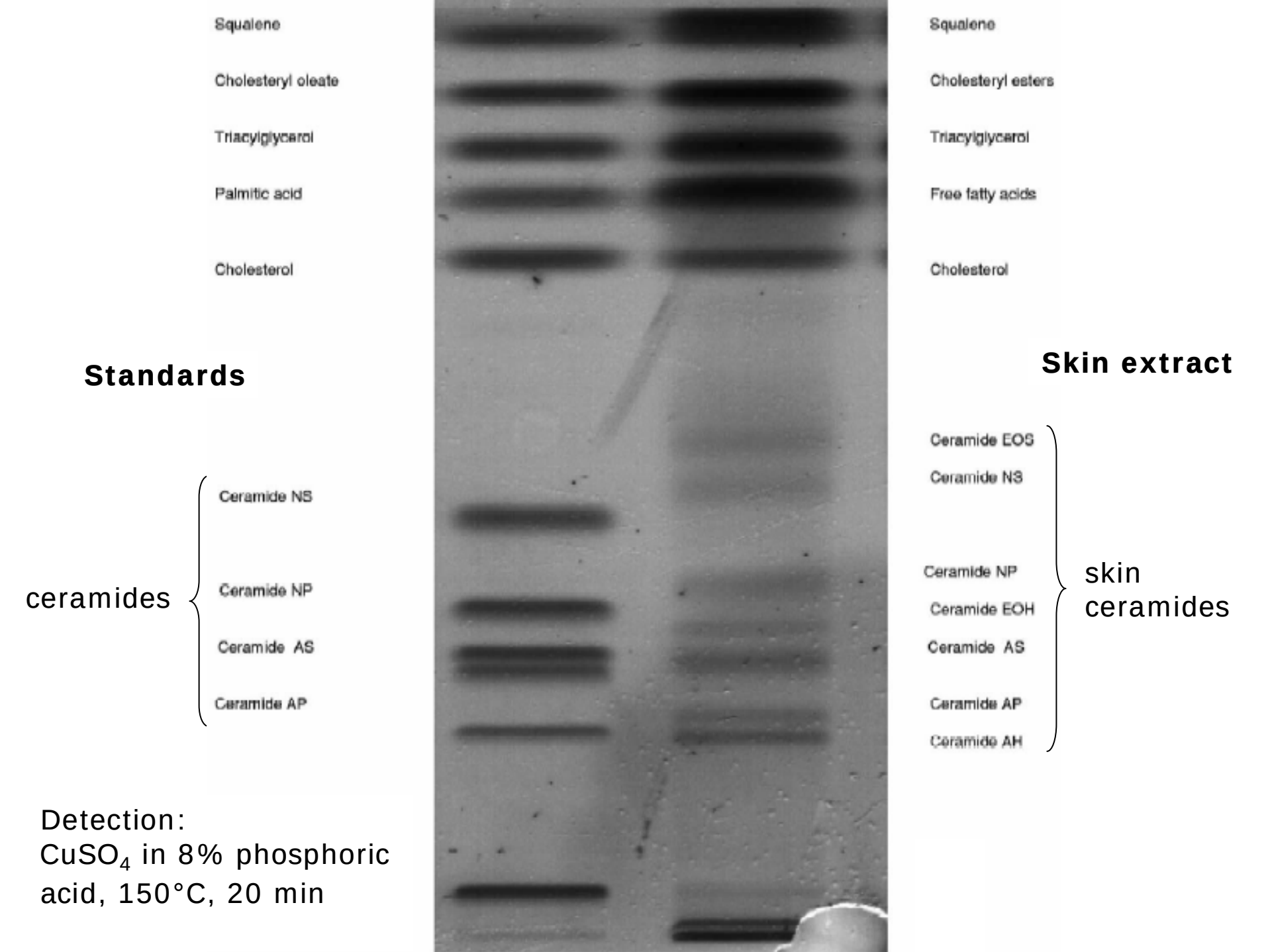

triacyl glycerides

Do ceramide profiles
of *Stratum corneum*
differ between
patients with
neurodermitis
and psoriasis
from normal skin lipids?

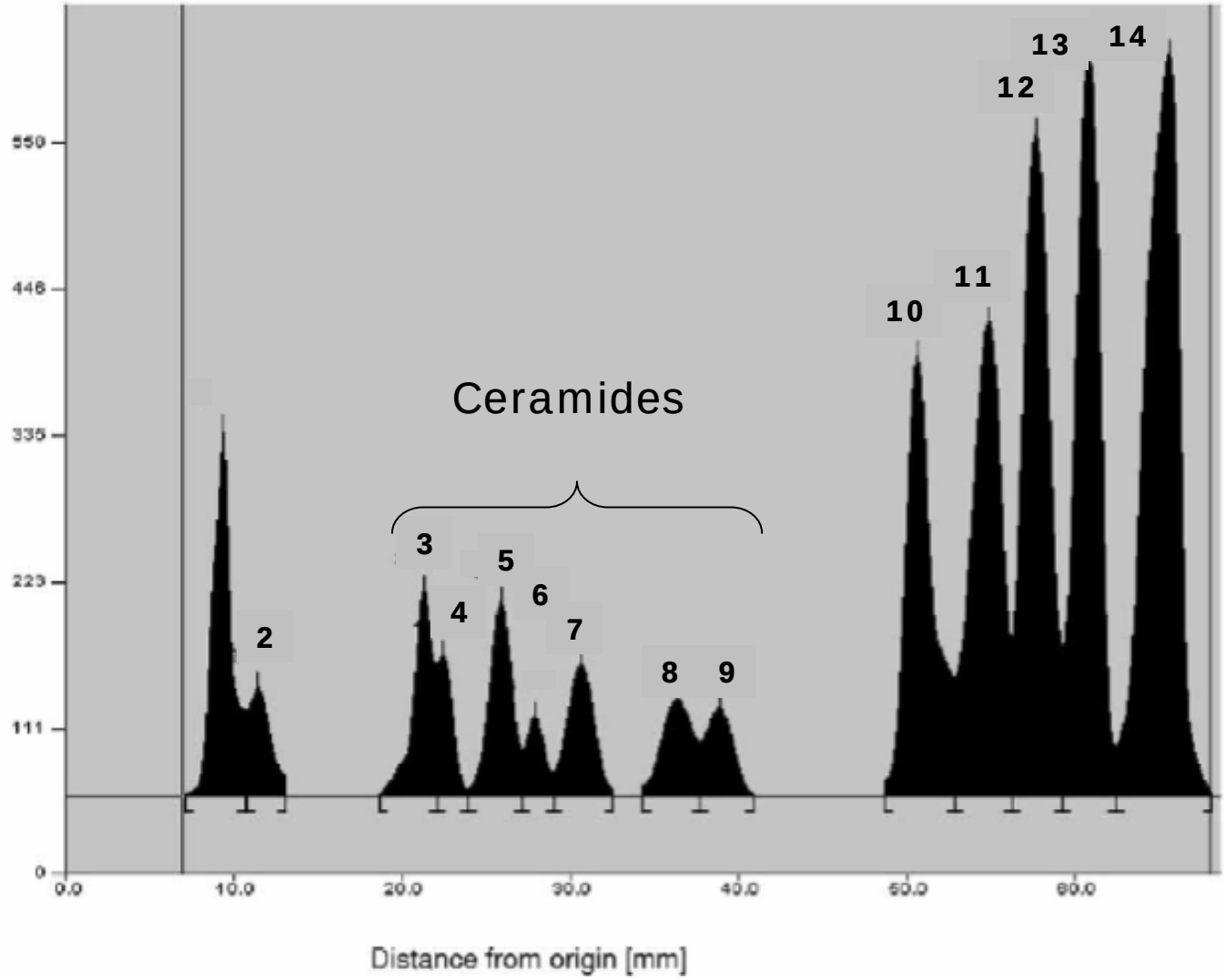


-> Separation and quantitation required!


Mobile phase composition % (v/v)
Distance from the origin/mm
Steps
AMD
Separation
Ethanol
Acetone
Chloroform
Ethylacetate
n-Hexane



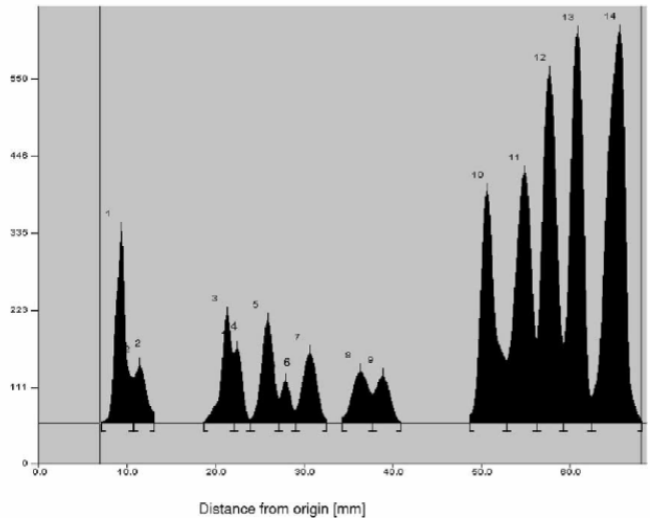

Detection:
$CuSO_4$ in 8% phosphoric acid, 150°C, 20 min




2 cholesterol-3-sulfate
3 Ceramide AH, 4 Ceramide AP, 5 Ceramide AS, 6 Ceramide EOH, 7 Ceramide NP
8 Ceramide NS, 9 Ceramide EOS
10 cholesterol, 11 fatty acids, 12 triacylglycerol, 13 cholesteryl esters, 14 squalene

# Characteristics and advantages of AMD separation

- Normal phase HPLC: less robust to matrix components from biological lipid extracts
- Reversed-phase LC: different selectivity not allowing ceramide class separation
- Detection problems resulting from low UV absorption




Farwanah, Neubert, Zellmer, Raith (2002) Improved procedure for the separation of major stratum corneum lipids by means of automated multiple development thin-layer chromatography. J. Chromatography B 780, 443-450

# Summary

AMD separation on HPTLC silica gel plates

- high versatility
- easy chemical derivatisation
- applicable to compounds with high polarity, high structural similarity

✎ not susceptible to matrix contamination

## Ceramide nomenclature

- EOS: ester-linked fatty acids, ω-OH fatty acids and sphingosines
- EOH: ester-linked fatty acids, ω-OH fatty acids and 6-hydroxysphingosine
- NS: non-OH fatty acids and sphingosines
- NP: non-OH fatty acids and phytosphingosines
- AS: α-OH fatty acids and sphingosines
- AP: α–OH fatty acids and phytosphingosines
- AH: α–OH fatty acids and 6-hydroxysphingosine

In accordance with
Motta, S. et al. (1993) *Biochimica et Biophysica Acta,* 1182: 147-151