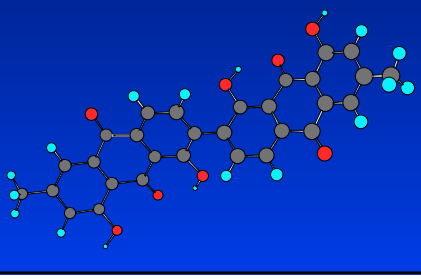
Distribution and Biological Activity of Anthraquinones in soil



Kobe University
Takeshi Suzuki

Contents

What is anthraquinones ?

Origin of anthraquinones

·Known more than 600 species as a Secondary metabolites of plants, microorganisms, lichens and insects
·Chemical synthesis

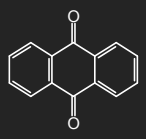
Uses of anthraquinones

As pigments

Dye of textile
Food additive
Hair dye
cosmetics
paints
smoke curtain

As medicines

Component of anti-carcinogecitic a crude drug
Antitumor chemicals
Anitbiotics
diuretics
laxative

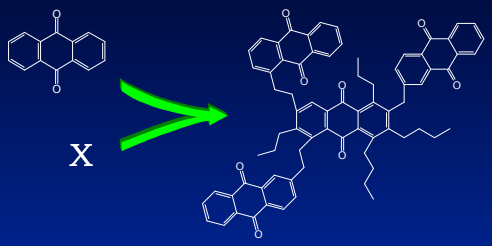


recently

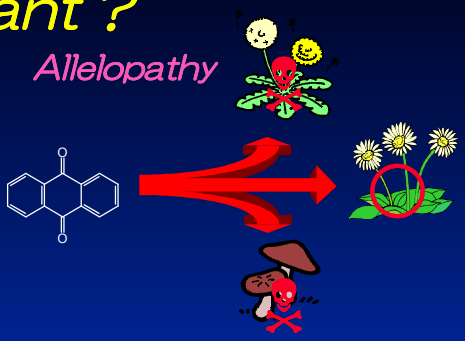
Carcynogecity and toxicity to human beeing

Why study of anthraquinons in soil is important ?

Constituent of soil humics ?

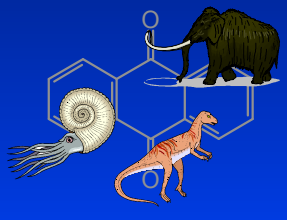


Allelopathy

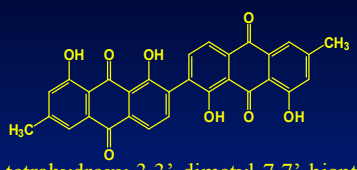


Complex capability with metal

Index chemicals of time

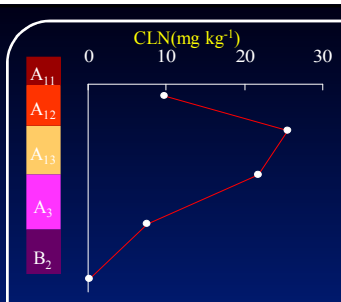


Review of study of Anthraquinones
-concerning chrysotalunin



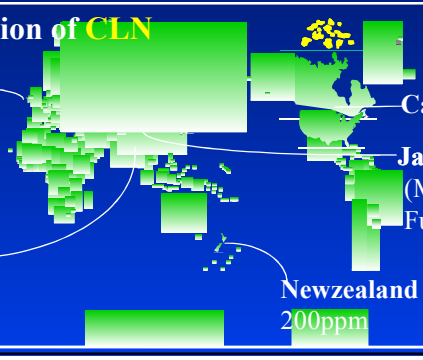
1,1',8,8'-tetrahydroxy-3,3'-dimetyl-7,7'-bianthraquinone

CLN



Distribution of CLN in Makino soil profile

Geographical distribution of CLN



Ireland (McGrath, 1967)

Canada (McGrath, 1972)

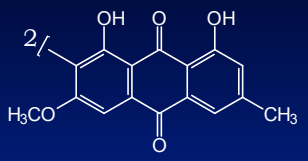
Japan 90 site (Matui & Kumada, 1974; Fujitake et al., 1990,1991)

Nepal (Suzuki, 1997)

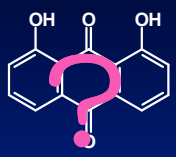
Newzealand (Foo & Tate, 1977) 200ppm

Review of study of Anthraquinones in soil
—Antraquinone without chrysotalunin

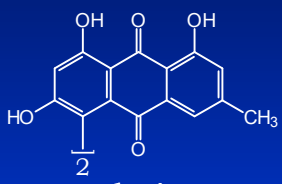
	R_1	R_2
chrysazin	H	H
chrysophanol (CPL)	CH_3	H
physcion (PYS)	CH_3	OCH_3
emodin	CH_3	OH



7,7'-biphyscion (7BP)



unknown compound X_1 X_2



skyrin

Main anthraquinones in soil are CLN, CPL, PYS, 7BP and two unidentified anthraquinones (Fujitake 1991).

Objective

There is no report AQ was extracted from a living thing

The synthetic pathway of anthraquinones in soil are different from the path way in *vivo*.

natural products chemical studies are need in field of soil science

- Identify chemical structure
- Clarify synthetic pathways
- Determine biological activity

Objective

clarification the synthetic pathway of anthraquinones in soil

- purification and identification of unknown anthraquinones in soil
- development of determination method of anthraquinones in soil
- clarification of the distribution pattern of anthraquinones in soil

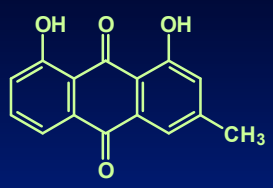
Clarification of the functions of anthraquinones in soil

Determination of biological activity by bioassay

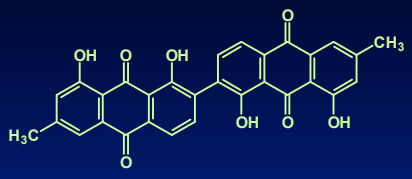
Contents

1. Introduction
2. *Purification and isolation of new anthra-quinones in soil*
3. Development of determination of main anthraquinones in soil
4. Distribution properties of anthraquinones in soil
5. Physiological activity of anthraquinone in aoil

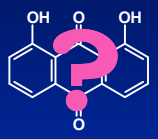
Main anthraquinones in soil



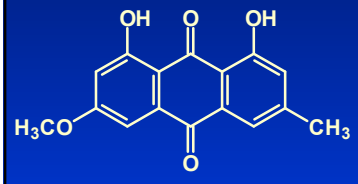
chrysophanol



chrysotalunin



unknown compound X_1 and X_2



physcion

7,7'-biphyscion

Soil sample

Hyogo Prefecture

Sampling ca. 20kg from surface layer

vegitation : *Sasa palmata*, *Castanea crenata*, *Quercus mongolica var. grosseserrata*, Pinus densiflora

Soil type : Andosol

air-dried soil (<2mm)

extracted with $CHCl_3$ in soxhlet apparatus

extracted solution

filtration

solution | pricipitate

shaken with 1N NaOH

NaOH phase | $CHCl_3$ phase

acidified by 3N HCl
shaken with EtOAc

EtOAc phase | H_2O phase

silica gel column chromat.
Hex:Bz(1:1-0:1), $CHCl_3$

extraction with hot $CHCl_3$

crude crystal

washing with H_2O,Me_2CO, EtOAc and hex.

Pig. A

Fr.1 | **Fr.2** | **Fr.3**

TLC on silica gel plates with Hex.:EtOAc:H_2O(9:1:saturated)

upper band | **lower band**

Pig.B | ***Pig.C***

Extraction and purification of anthraquinones（1）

Fr.2

TLC on silica gel plates with hex.:EtOAc:H_2O(9:1:saturated)

yellow band

recrystalization from EtOH and Hex.

yellow crystal

Pig.D

Fr.3

C_{18} column chromat. with 75% EtOH

Fr.3a

TLC on silica gel plates with hex.:EtOAc:H_2O(9:1:saturated)

red band

recrystalization from EtOH

crystal

Pig.E

Fr.3b

yellow band

recrystalization from EtOAc

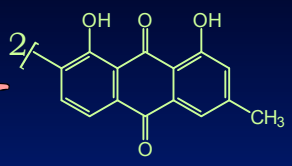
crystal

Pig.F

Extraction and purification of anthraquinones （2）

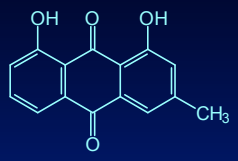
Pigments of corresponded to authentic sample of mass spectrum、Rf value of TLC、UV-Visible spectrum

Pig.A



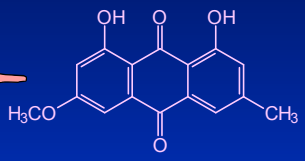
chrysotalunin

Pig.B



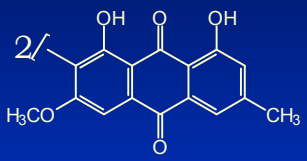
chrysophanol

Pig.C



physcion

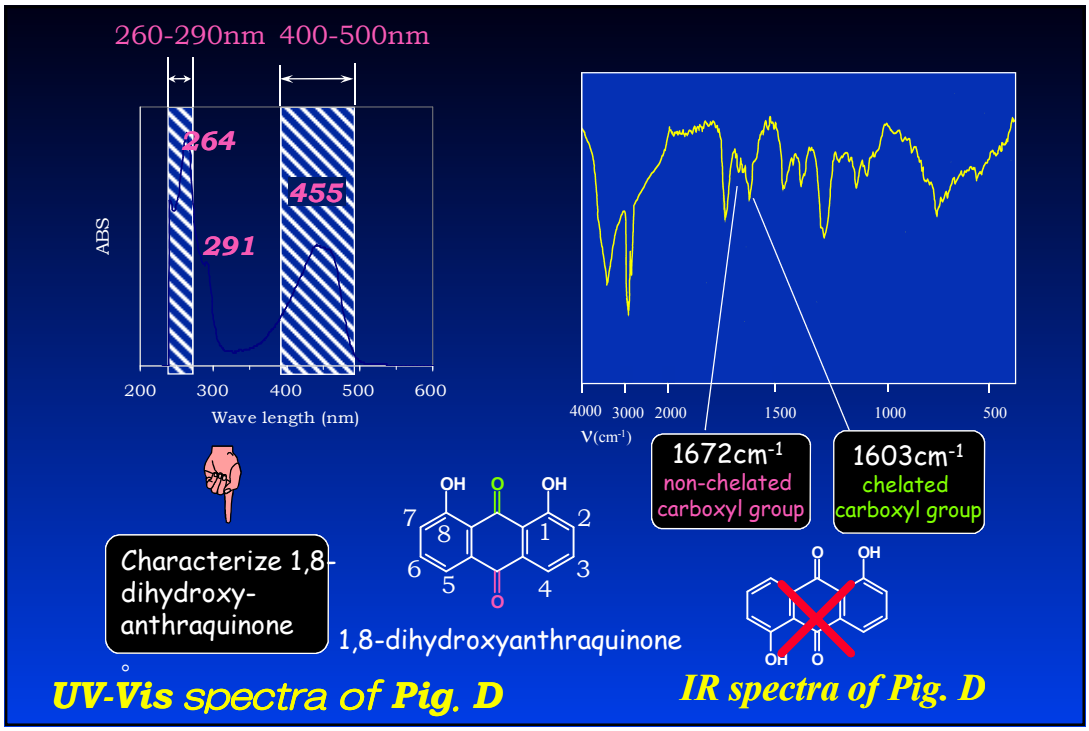
Pig.F

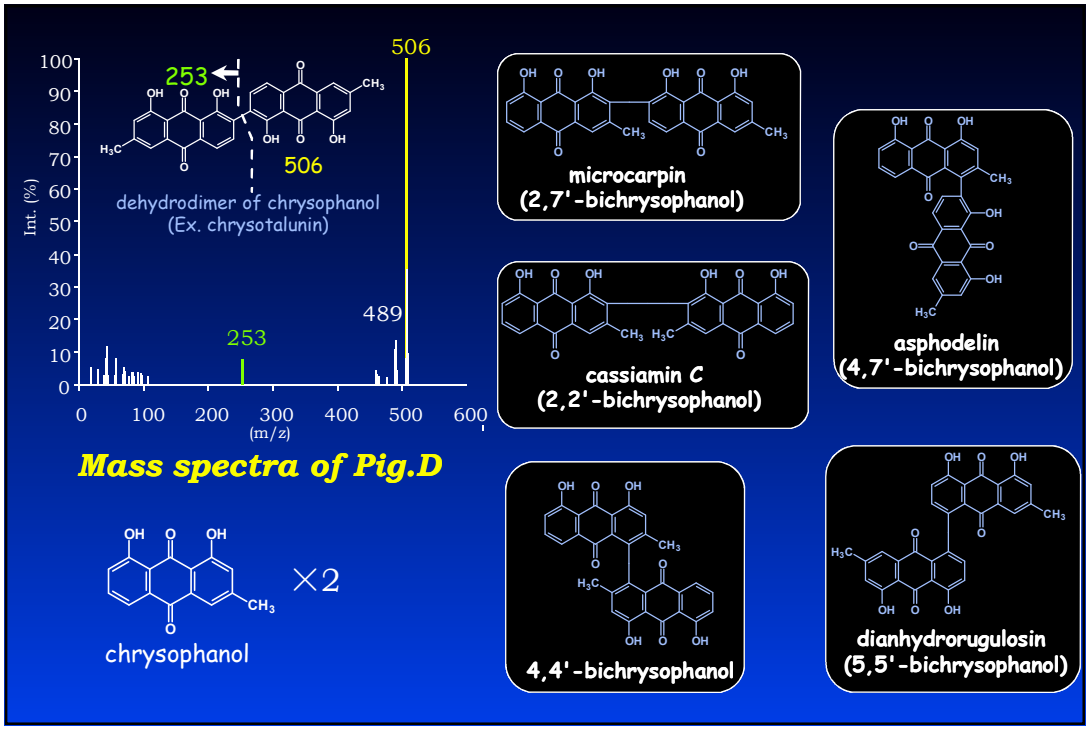


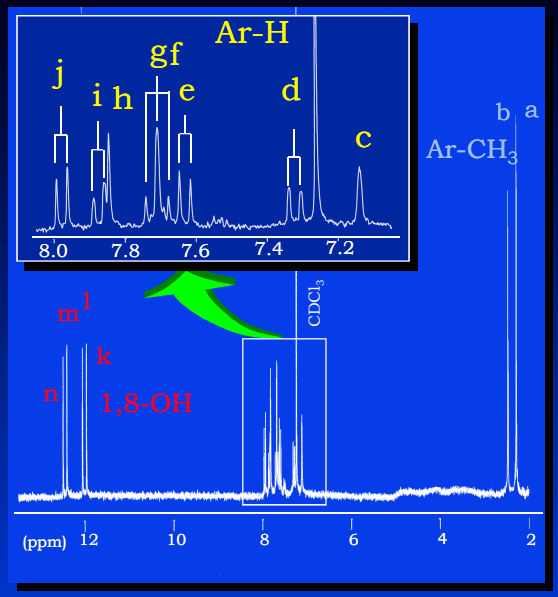
7,7'-biphyscion

Pig.D ?

Pig.E ?


260-290nm 400-500nm
264
455
291
ABS
200 300 400 500 600
Wave length (nm)
4000 3000 2000 1500 1000 500
ν(cm-1)
1672cm-1
non-chelated
carboxyl group
1603cm-1
chelated
carboxyl group
Characterize 1,8-dihydroxy-anthraquinone
1,8-dihydroxyanthraquinone
UV-Vis spectra of Pig. D
IR spectra of Pig. D

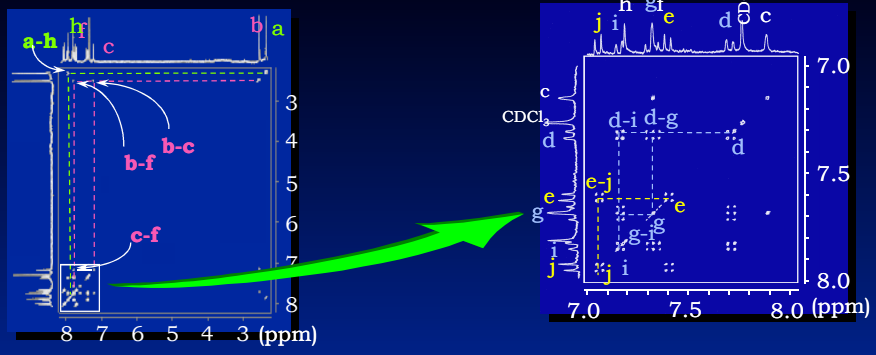

506
253
506
dehydrodimer of chrysophanol
(Ex. chrysotalunin)
Int. (%)
489
253
(m/z)
Mass spectra of Pig.D
chrysophanol
×2
microcarpin
(2,7'-bichrysophanol)
cassiamin C
(2,2'-bichrysophanol)
4,4'-bichrysophanol
asphodelin
(4,7'-bichrysophanol)
dianhydrorugulosin
(5,5'-bichrysophanol)

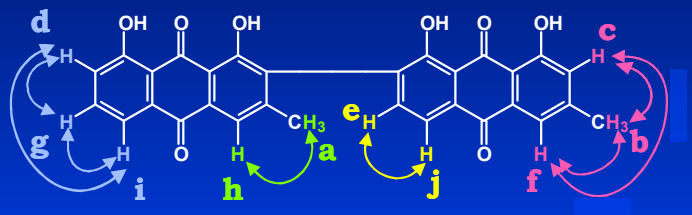


	Ar-CH_3	Ar-H	1,8-OH	*total*
number of signal	2	8	4	***14***

Microcarpin
(2,7'-bichrysophanol)

^{1}H-NMR spectra of Pig.D

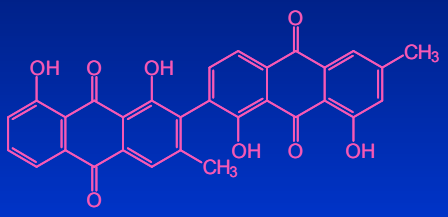




COSY spectra of Pig.D

λmax of UV-Vis spectrum

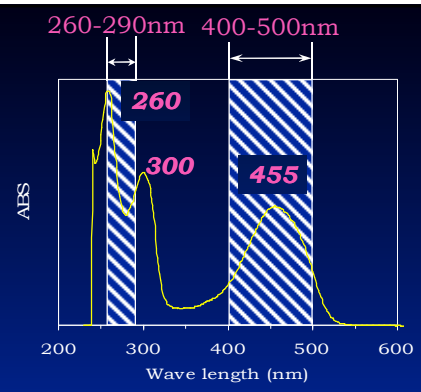
compound	λ_{max} ($CDCl_3$)
Microcarpin (MCP)	262, 290, 453
Pig.D	264, 291, 455
MCP-Ac	263, 347
Pig.D-Ac	263, 346



Microcarpin
(2,7'-bichrysophanol)

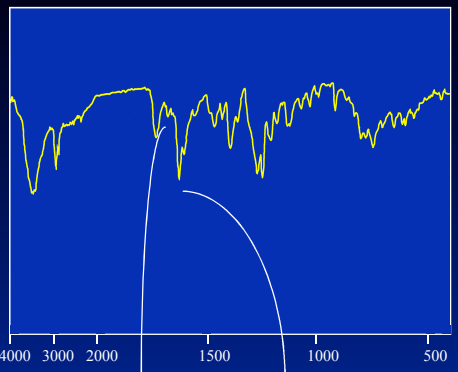
δ value of ^{1}H-NMR (ppm)

compound	Pig.D-Ac	MCP-Ac
Ar-CH_3	2.27s, 2.56s	2.23s, 2.53s
Ar-H	7.24bs 7.40dd (*J*=8,1.5) 7.58d(*J*=8) 7.77t (*J*=8) 8.04bs, 8.14s 8.23dd (*J*=8,1.5) 8.28d (*J*=8)	7.27bs 7.44dd (*J*=8,1.5) 7.59d (*J*=8) 7.80t (*J*=8) 8.06bs, 8.15bs 8.22dd (*J*=8,1.5) 8.26d (*J*=8)
O-Ac	2.15s, 2.17s 2.46s	2.04s, 2.07s 2.38s



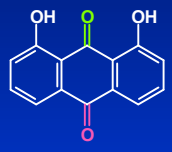
The peak of 1,8-dihydroxy-anthraquinone

UV-VIS spectra of Pig.E

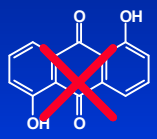


1672cm^{-1} non-chelated carboxyl group

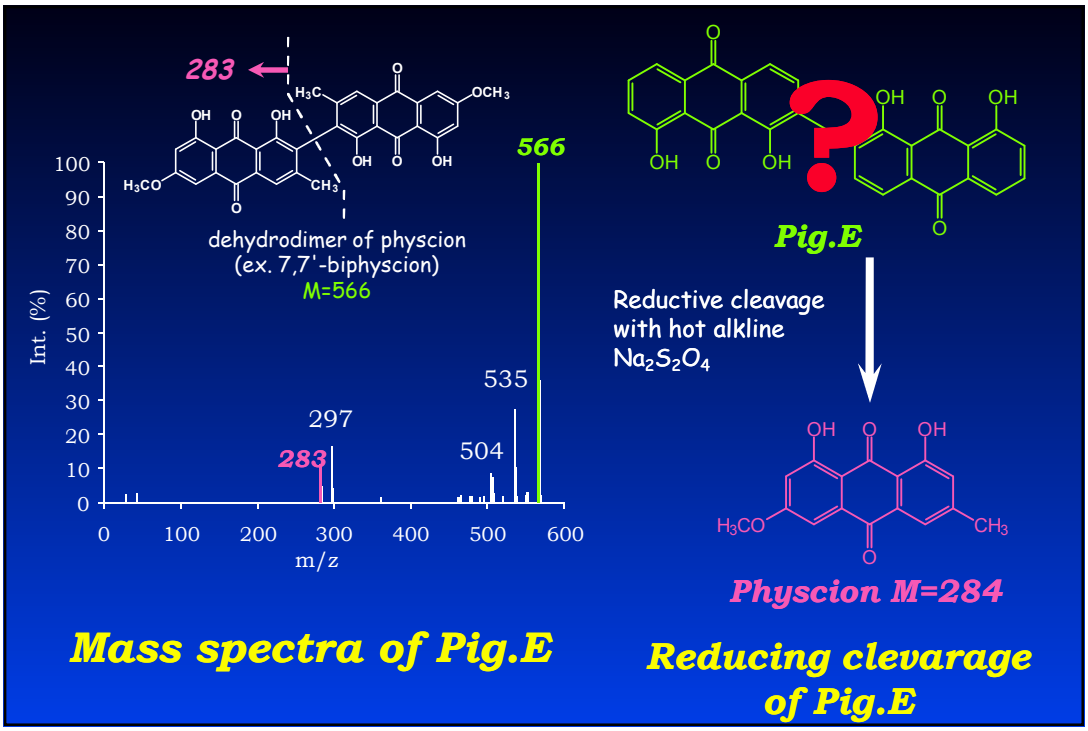
1603cm^{-1} chelated carboxyl group

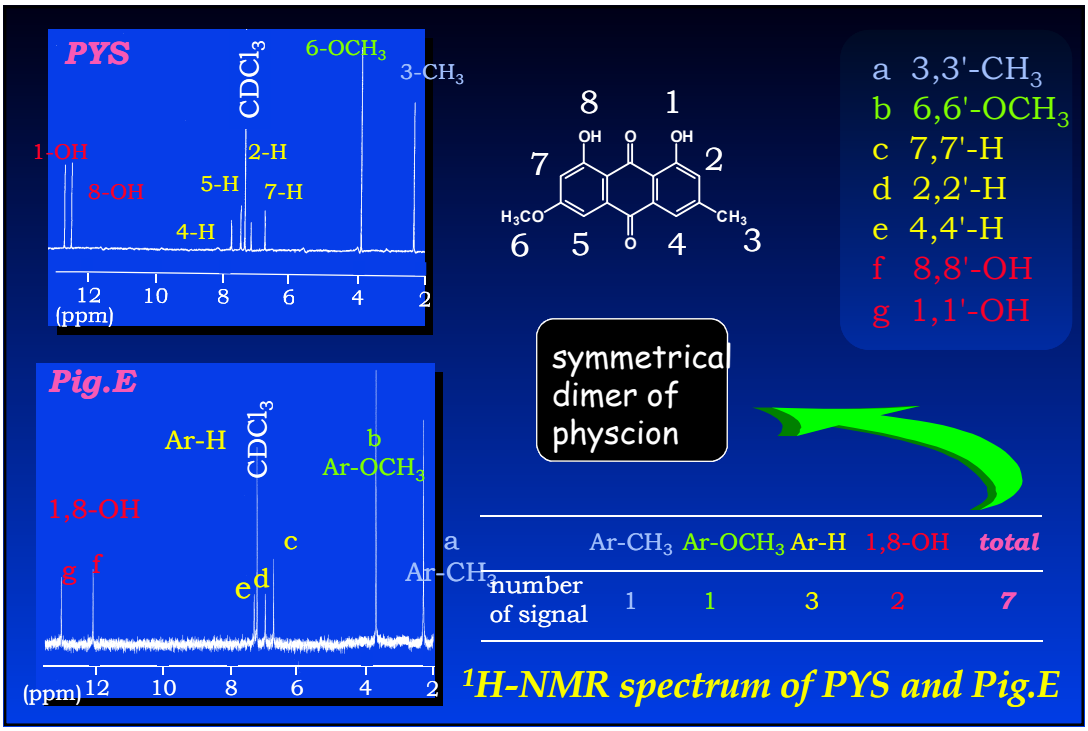


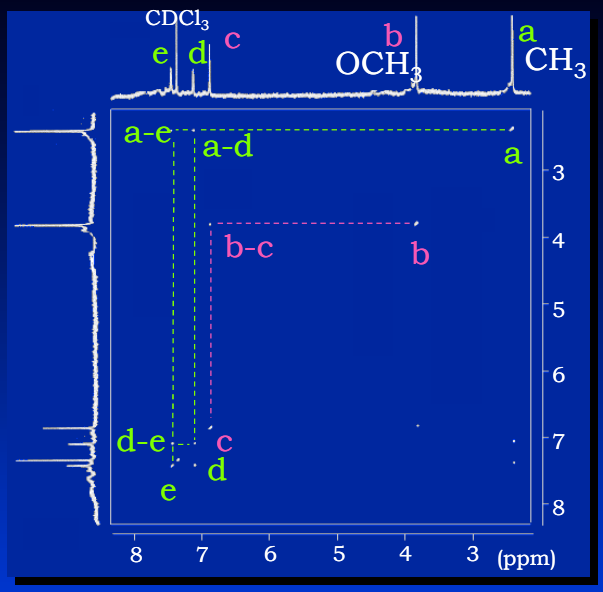
1,8-dihydroxyanthraquinone

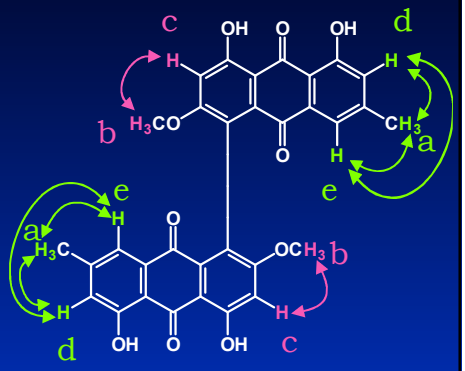


IR spectra of Pig.E

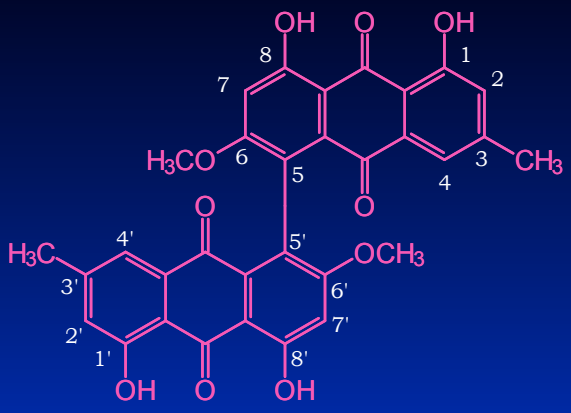

283
566
dehydrodimer of physcion
(ex. 7,7'-biphyscion)
M=566
Int. (%)
m/z
535
297
283
504
Pig.E
Reductive cleavage
with hot alkline
$Na_2S_2O_4$
Physcion M=284
Mass spectra of Pig.E
Reducing clevarage
of Pig.E


PYS
$CDCl_3$
6-OCH_3
3-CH_3
1-OH
2-H
8-OH
5-H
7-H
4-H
(ppm)
Pig.E
Ar-H
$CDCl_3$
b
Ar-OCH_3
1,8-OH
c
a
Ar-CH_3
g
f
e
d
(ppm)
a 3,3'-CH_3
b 6,6'-OCH_3
c 7,7'-H
d 2,2'-H
e 4,4'-H
f 8,8'-OH
g 1,1'-OH
symmetrical
dimer of
physcion
Ar-CH_3 Ar-OCH_3 Ar-H 1,8-OH total
number
of signal 1 1 3 2 7
^{1}H-NMR spectrum of PYS and Pig.E





Cosy spectrum of Pig.E



5,5'-biphyscion (named hinakurin: HKR)

Chemical structure of Pig.E

Origin of main anthraquinones in soil

	Higher plants	Microorganism
CPL	Rhamnus, Rumex, Cassia, Polygonam, Phaseolus, Rubia, Elatostema, etc.	Many fungi, many lichen
PYS	Rhamnus 、Zingiber 、liliaceousetc.	fungi：*Alternaria porri, penicillium charlesii* etc. Many fungi, many lichen
CLN	*nothing*	*nothing*
7BP	nothing	***Dermocybe cinnamomeolutea*** ***Tricholoma equestre***
MCP	*Asphodelus microcarpas* (liliaceous) *Asphodeline* (liliaceous) (only in south Europe)	nothing
HKR	nothing	nothing

The dimer antraquinones in soil (CLN, 7BP, MCP, HKR) are rare compound in nature

Conclusion in this chapter

- Isolate and identify chrysotalunin, chryso-phanol, physcion, 7,7'-biphyscion, micro-carpin (2,7'-bichrysophanol)、hinakurin (5,5'-biphyscion)
- microcarpin is new compound in soil Hinakurin is new compound in nature
- Dimer of anthraquinones are rare in nature, but predominant in soil

Contents

1. Introduction
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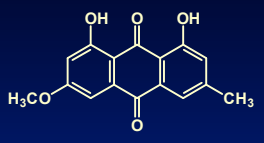
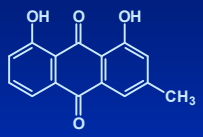
main anthraquinones in soil



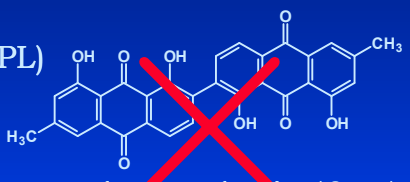
physcion (PYS)

7,7'-biphyscion (7BP)

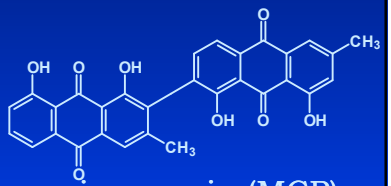
hinakurin (HKR)
(5,5'-biphyscion)



chrysophanol (CPL)



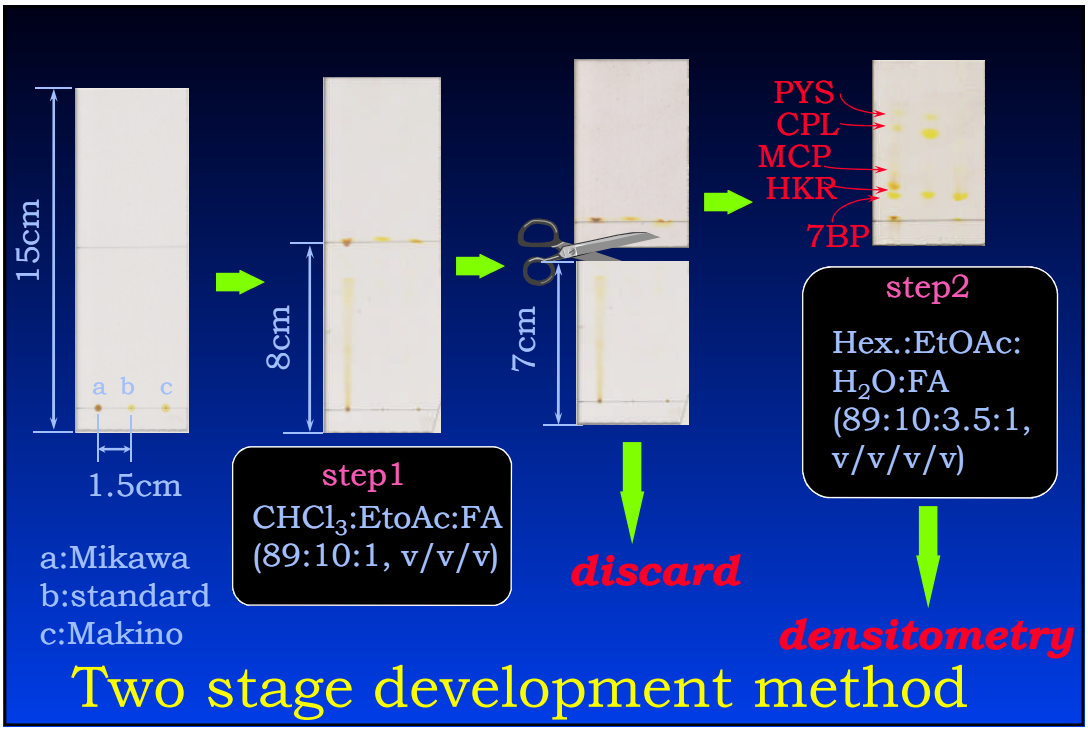
chrysotalunin (CLN)
(7,7'-bichrysophanol)



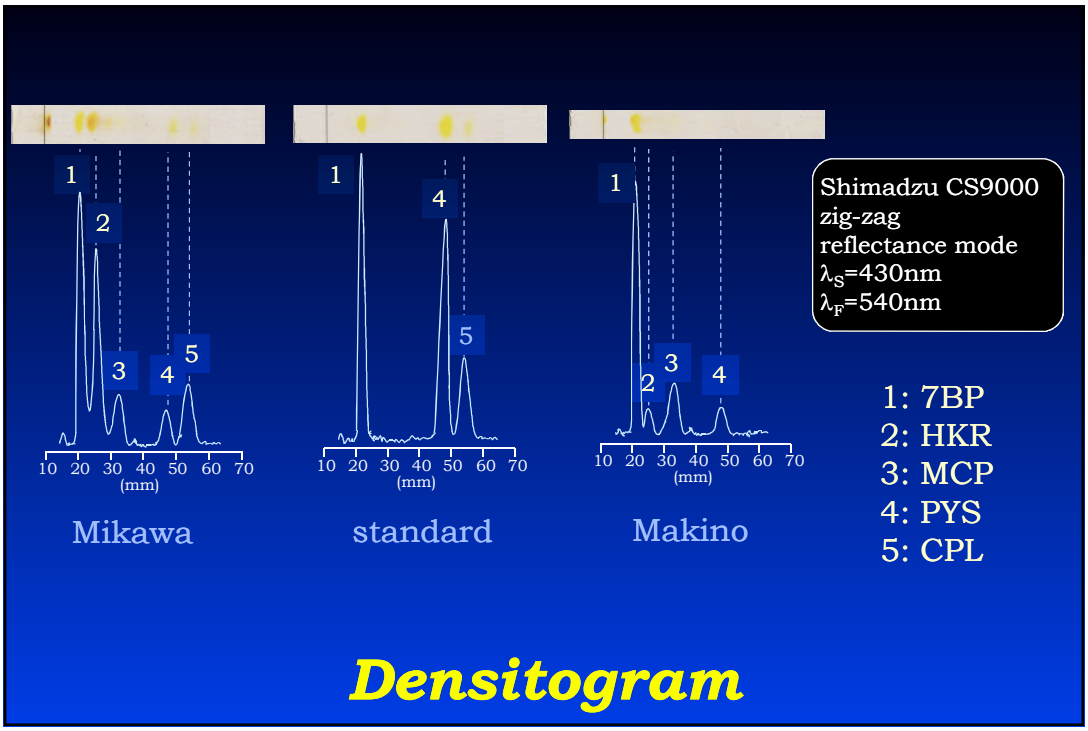
microcarpin (MCP)
2,7'-bichrysophanol

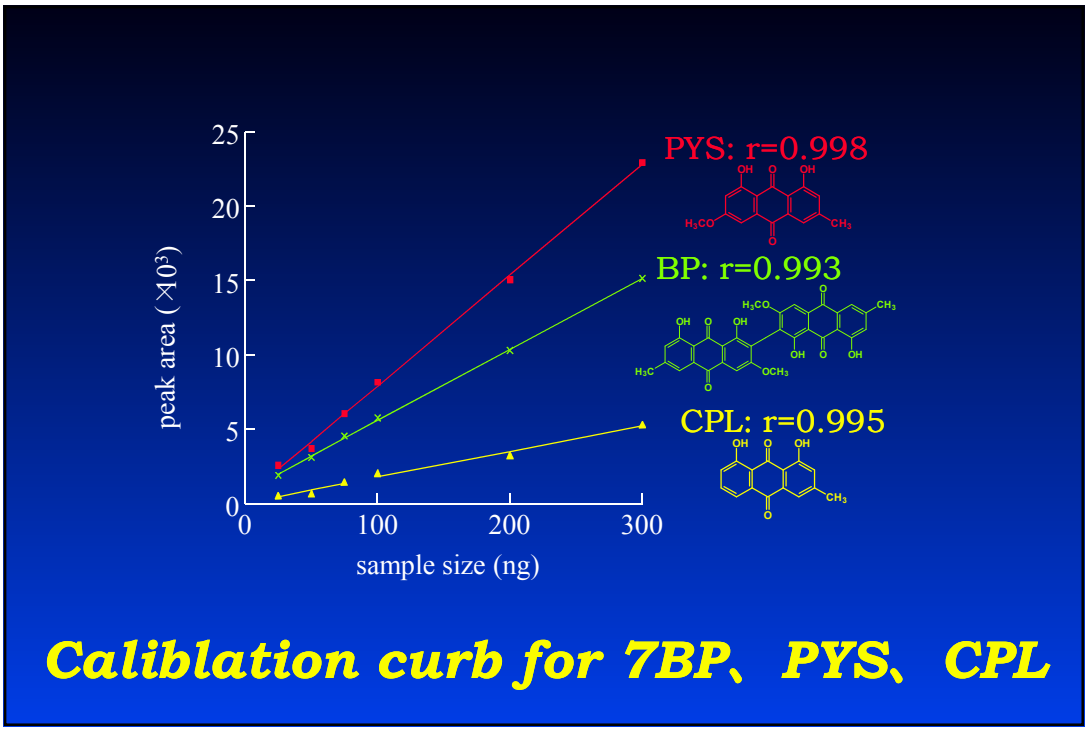
Which method do use for determination of anthraquinones in soil?

~~HPLC~~	Impuriteis are irreversisively absorbed on stationary phase
~~GC~~	AQs do not vaporize Impurities are irreversisively absorbed on stationary phase Low sensitivity
~~One dimensional TLC~~	AQs can't be completely separated because of many impurities
Two dimensional TLC	Quantitative determination is not simplyfied The number of sample at one development is too low
Multi development (two stage development) TLC	Quantitative determination is same as well as 1D TLC The number of sample is same as well as 1d TLC The Separability is same as well as 2d TLC



Two stage development method


1
2
3
4
5
10 20 30 40 50 60 70
(mm)
Mikawa
standard
Makino
Shimadzu CS9000
zig-zag
reflectance mode
λS=430nm
λF=540nm
1: 7BP
2: HKR
3: MCP
4: PYS
5: CPL
Densitogram

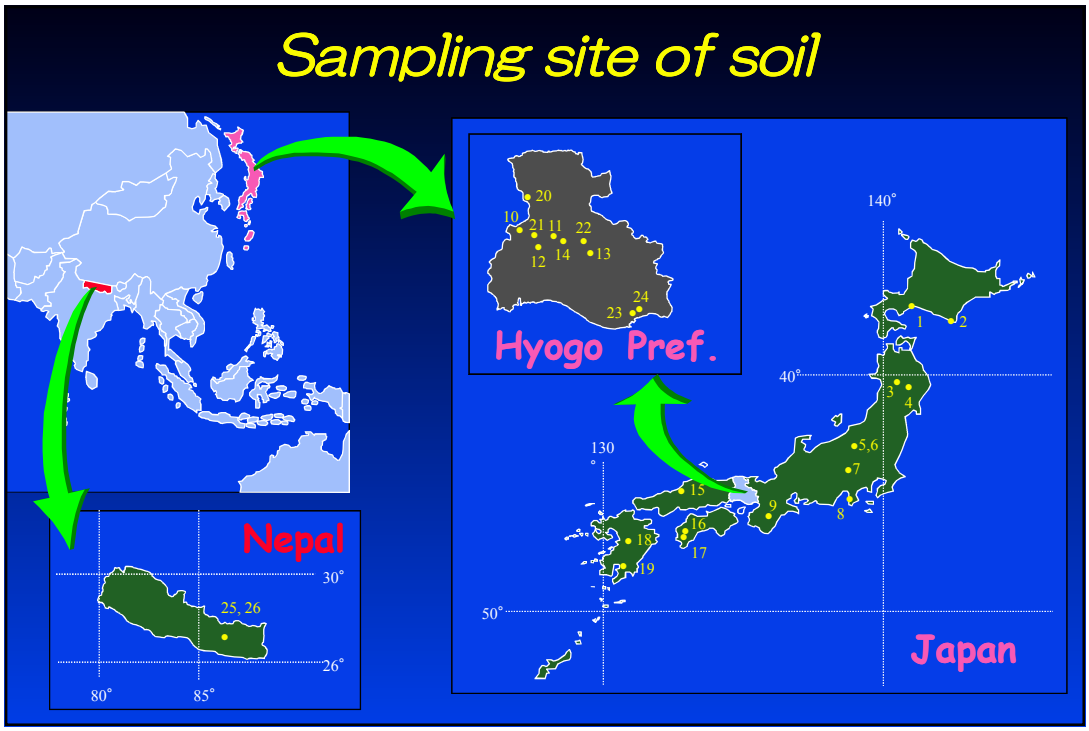

peak area (×10³)
sample size (ng)
0
5
10
15
20
25
0
100
200
300
PYS: r=0.998
BP: r=0.993
CPL: r=0.995
Caliblation curb for 7BP、PYS、CPL

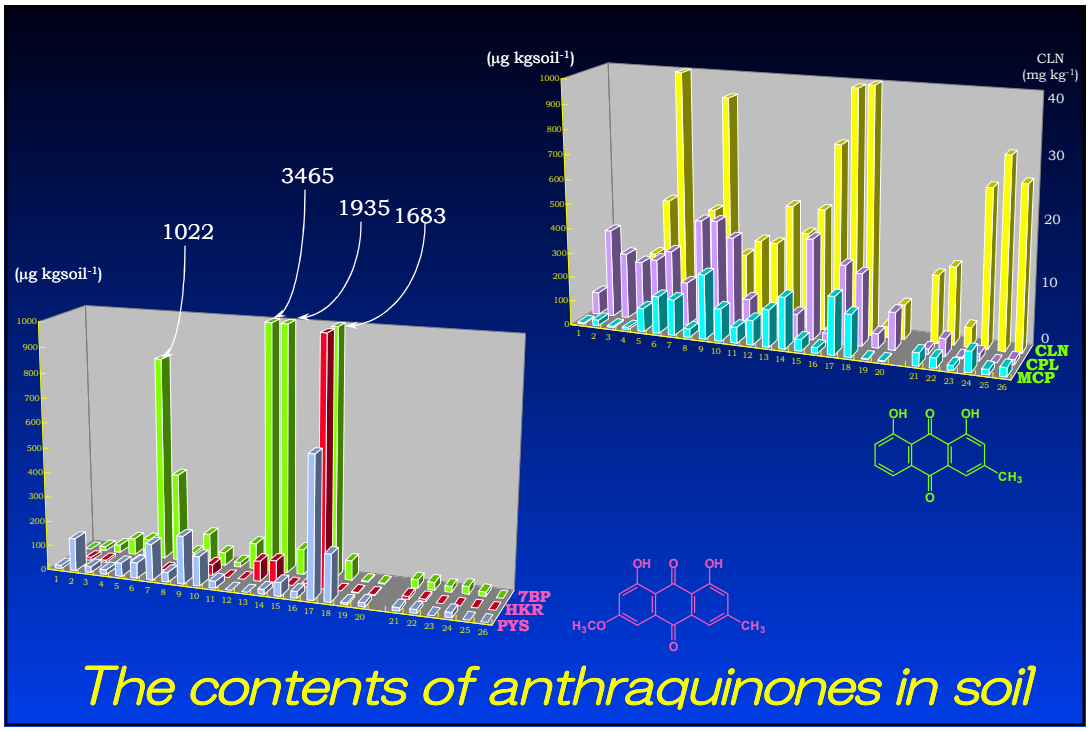
Conclusion in this chapter

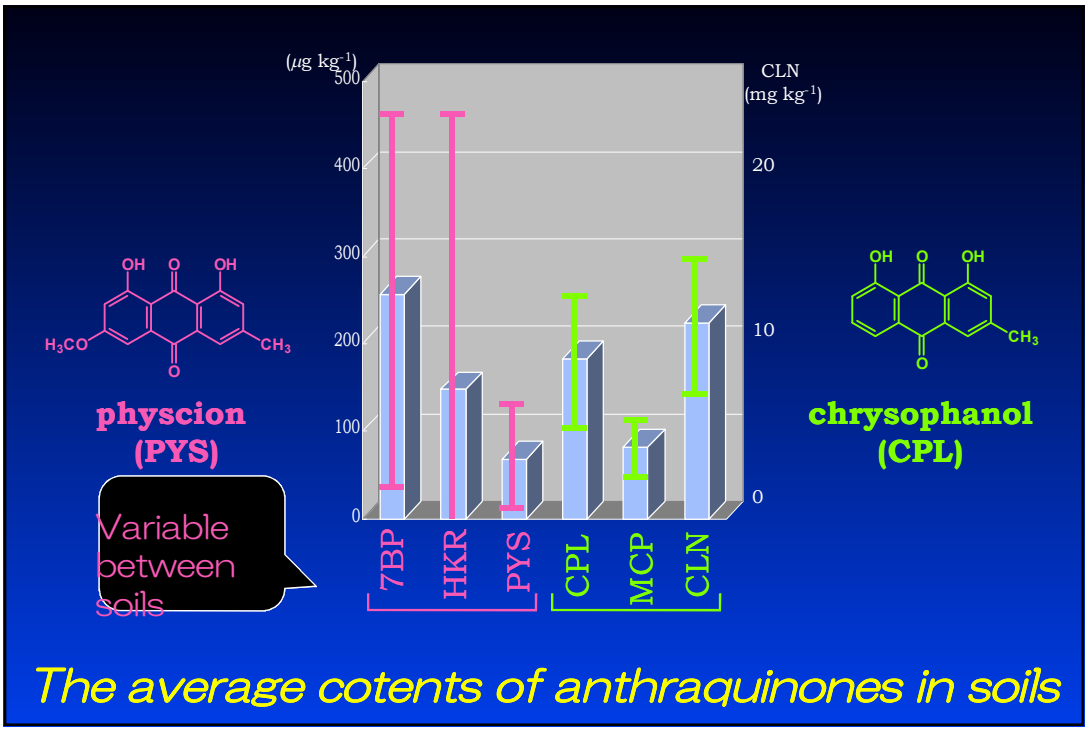
Five anthraquinones in soil（7BP、HKR、MCP、PYS、CPL）can be determine by two stage development TLC with scaning densitmetry

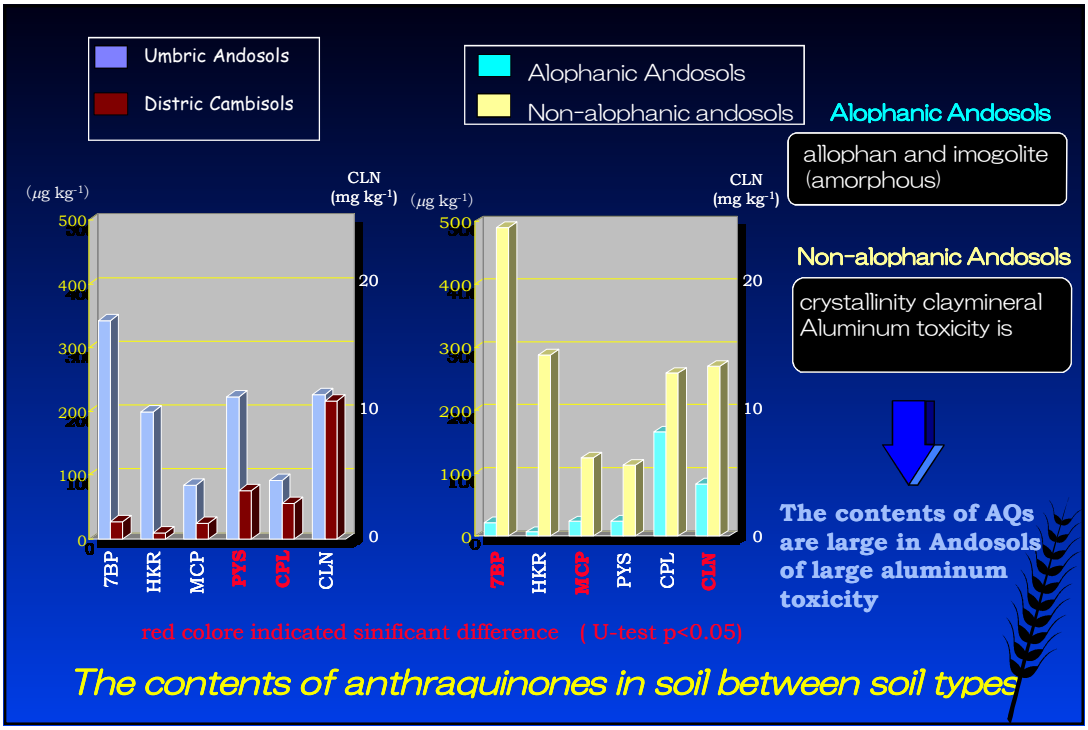
Contents

1. Introduction
2. Purification and isolation of new anthraquinones in soil
3. Development of determination of main anthraquinones in soil
4. *Distribution properties of anthraquinones in soil*
5. Physiological activity of anthraquinone in soil


Sampling site of soil
Hyogo Pref.
Nepal
Japan
140°
40°
130
50°
30°
26°
80°
85°
25, 26

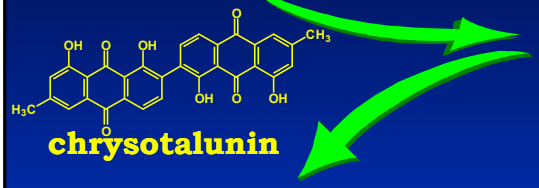

(μg kgsoil-1)
1022
3465
1935
1683
7BP
HKR
PYS
CLN
(mg kg-1)
CLN
CPL
MCP
OH
O
CH3
H3CO
The contents of anthraquinones in soil


(μg kg⁻¹)
CLN (mg kg⁻¹)
500
400
300
200
100
0
20
10
0
7BP
HKR
PYS
CPL
MCP
CLN
physcion (PYS)
chrysophanol (CPL)
Variable between soils
The average cotents of anthraquinones in soils


Umbric Andosols
Distric Cambisols
Alophanic Andosols
Non-alophanic andosols
Alophanic Andosols
allophan and imogolite (amorphous)
Non-alophanic Andosols
crystallinity claymineral
Aluminum toxicity is
The contents of AQs are large in Andosols of large aluminum toxicity
red colore indicated sinificant difference (U-test p<0.05)
The contents of anthraquinones in soil between soil types

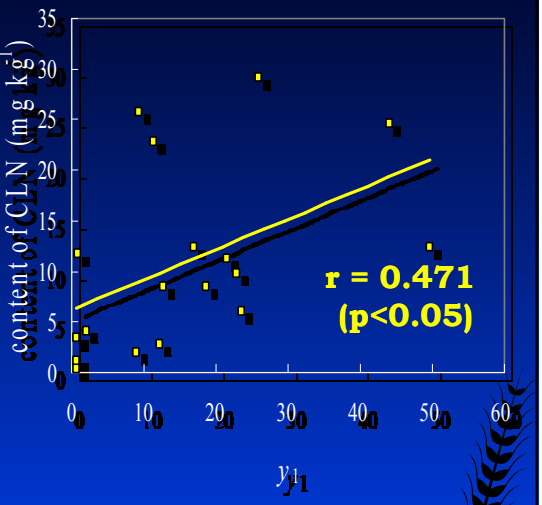
Exchange acidty（y_1）is used as a index of aluminum toxicity（Saigusa et al. 1980）。

Correlation of y_1 and contents of anthraquinones

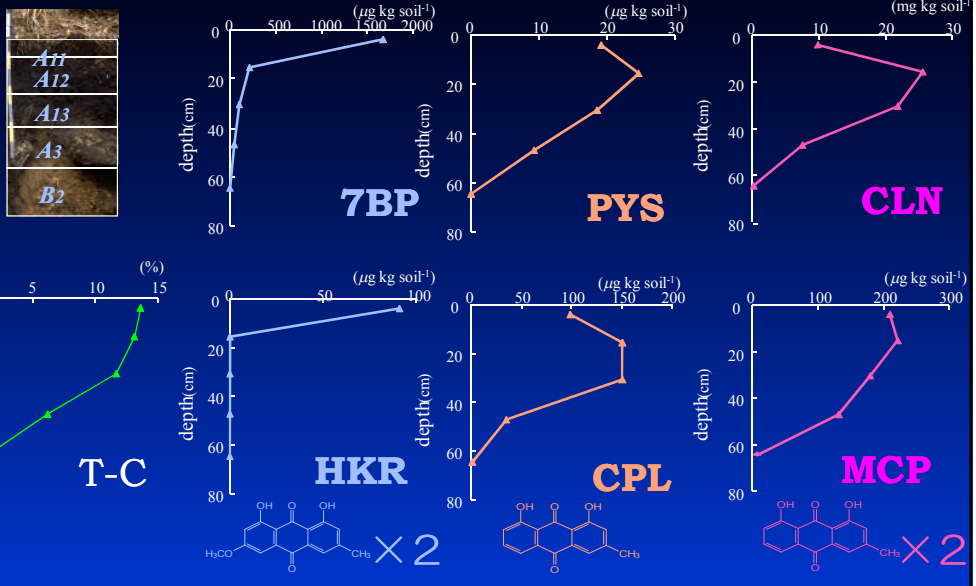


chrysotalunin

CLN is Large contents in large aluminum toxcitic Andosol



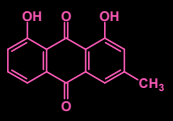
Correlation of y_1 and contents of anthraquinones

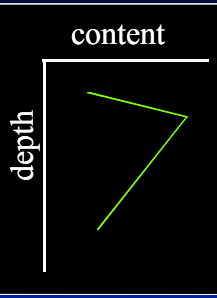


Vertical distribution of AQs in MKN soil profile

Vertical distribution of AQs in Andosols profiles

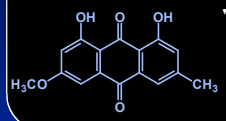
CLN
MCP
CPL

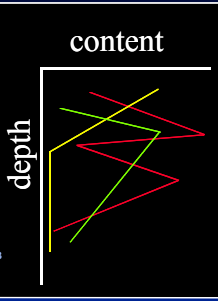




The peak of contents is the middle of surface horizon in all profiles

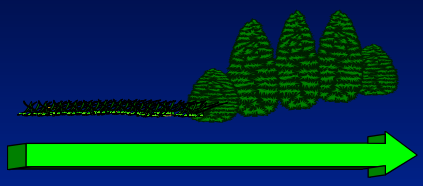
7BP
HKR
PYS





The contents peak is different in every soil profile

Succession of grass land to forest



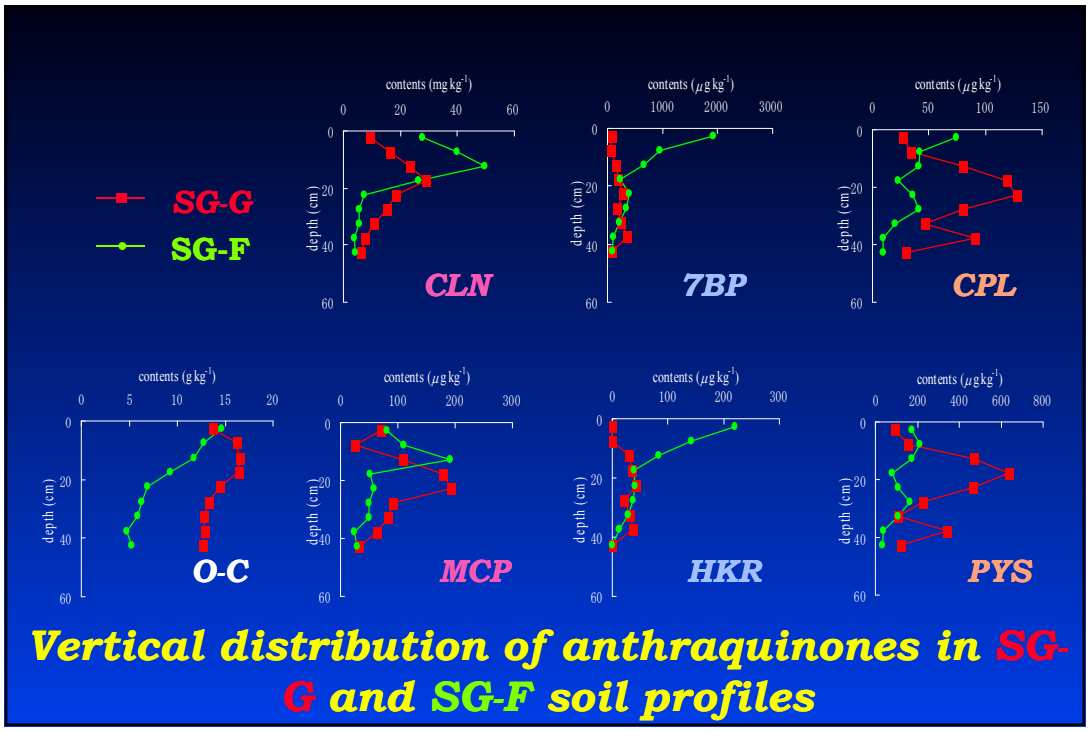
Difference of anthraquinone？

SG-G

Maintain grassland by cutting grass

SG-F

Forest after 40 year



discussion of this chapter

compound	horizontal	vertical	grass or forest
CLN, MCP	Predominantly occuring in all soil. Not variable between soils	content / depth	GRASS ≦ FOREST
CPL, PYS, HKR, 7BP	Occuring in almost soil variable between soils	content / depth	GRASS > FOREST; GRASS < FOREST

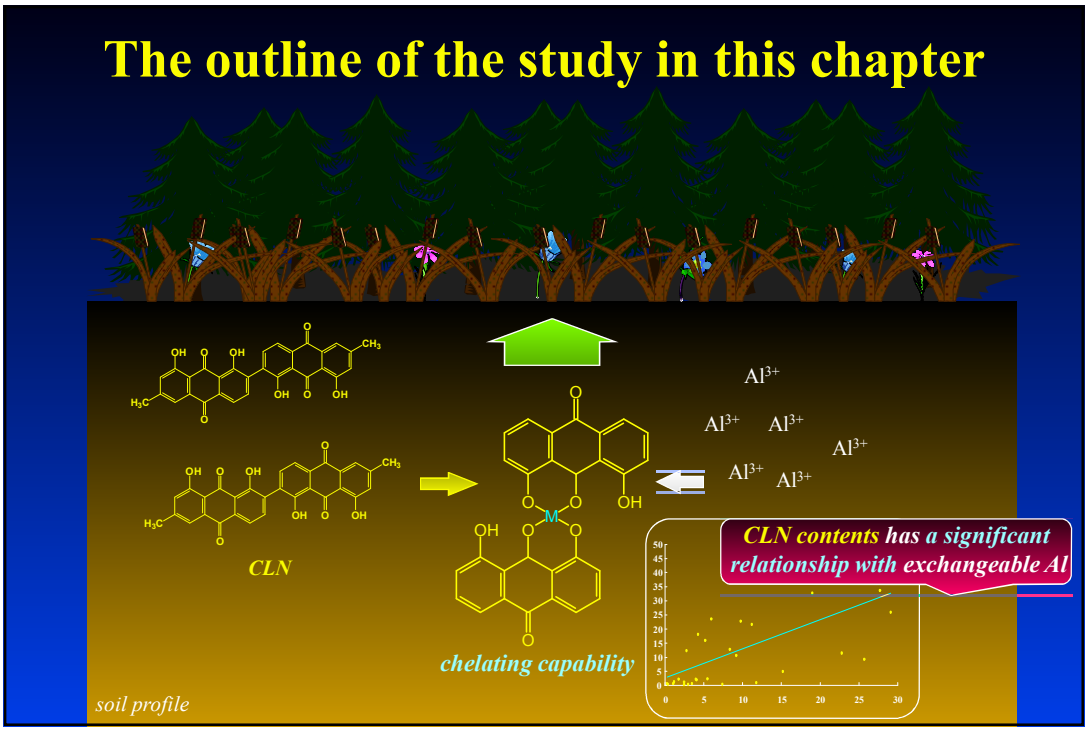
CPL and its dimers and PYS and its dimers are made different synthetic pathway in soil

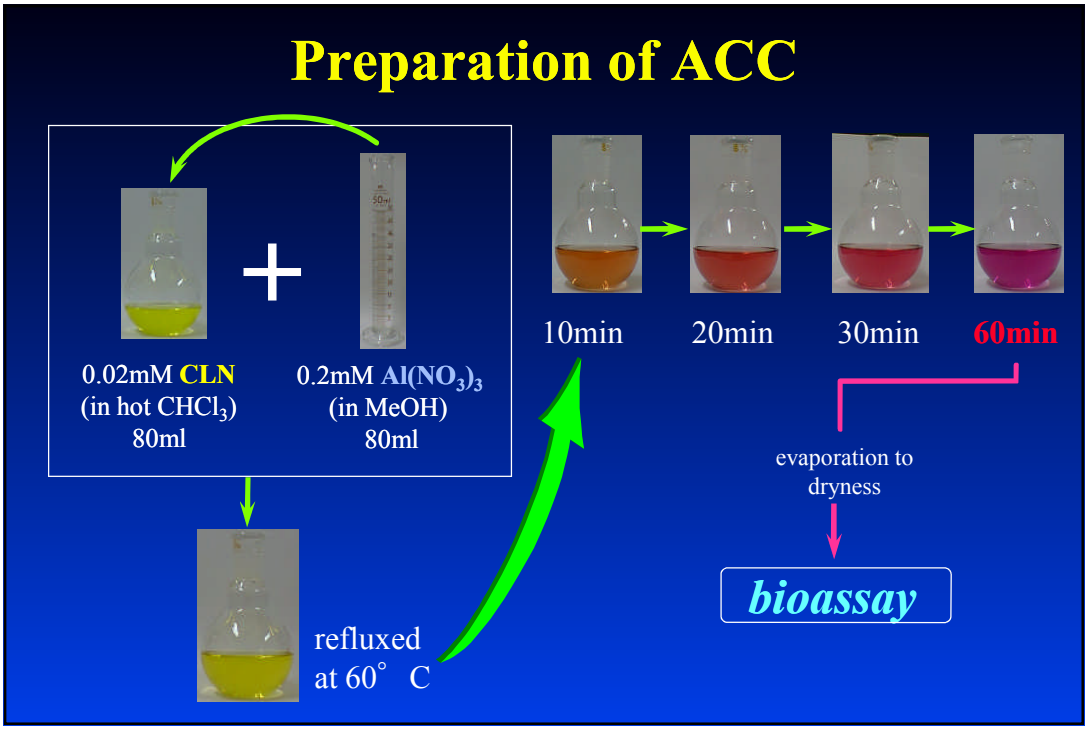
Dimeric AQs are rear in nature but predominatnt in soil

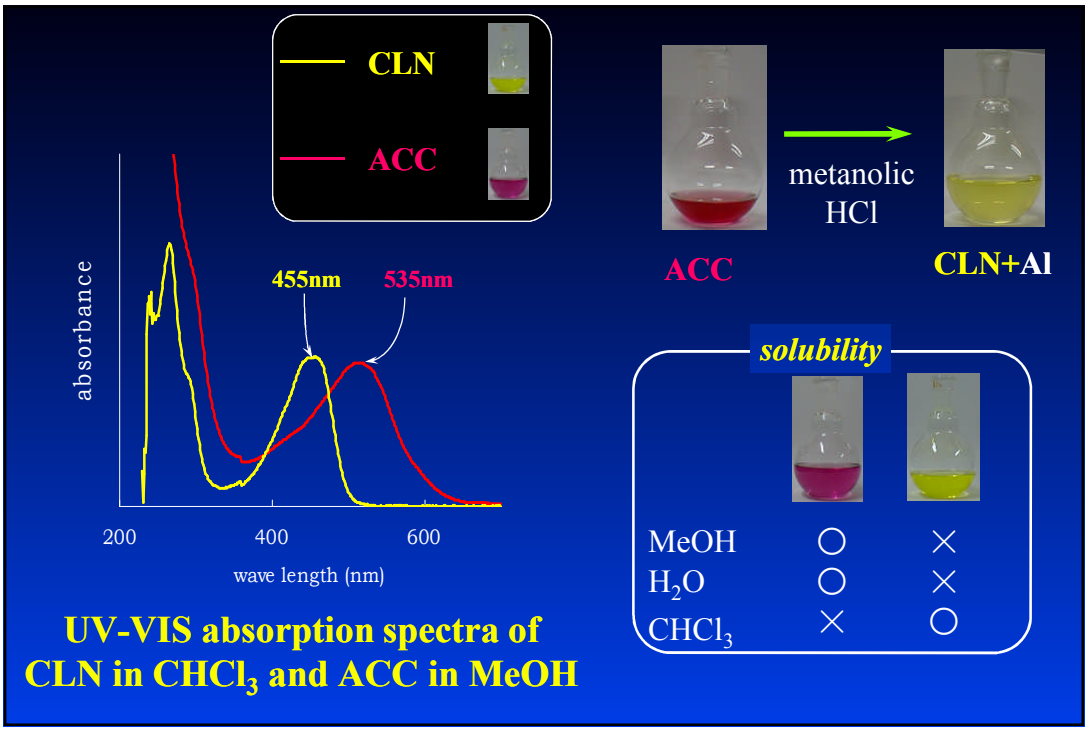
Monomeric AQs decrease and dimeric AQs are increase accordingly forestration

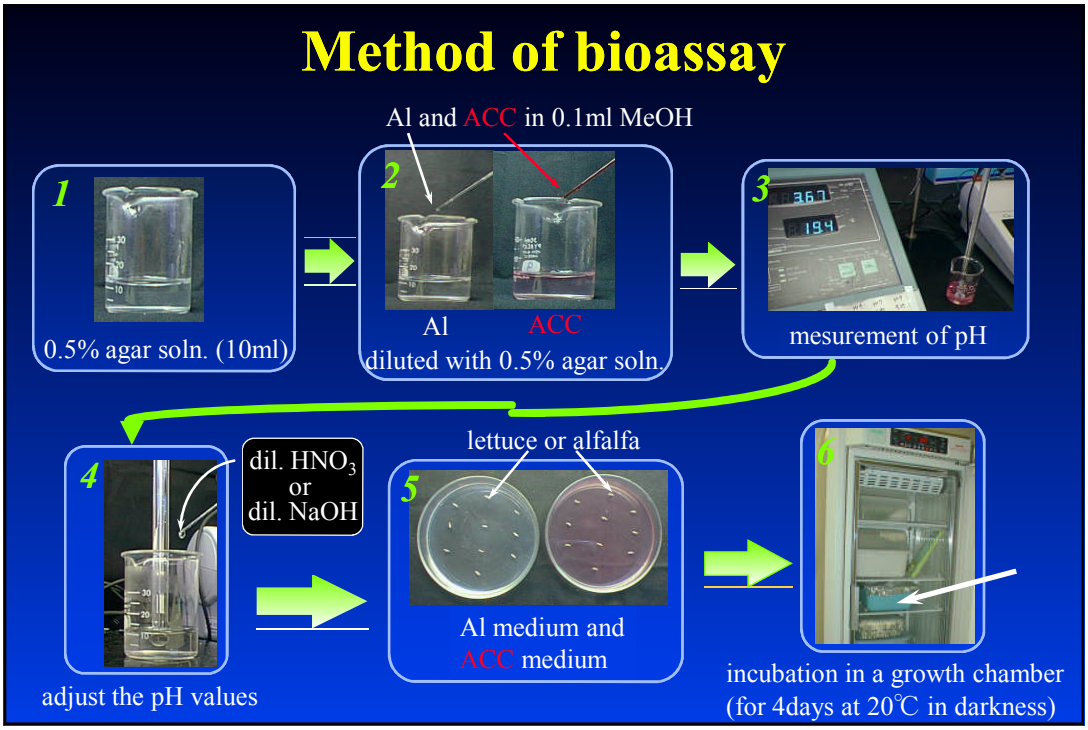
Monomeric AQ are predominant occuring in nature

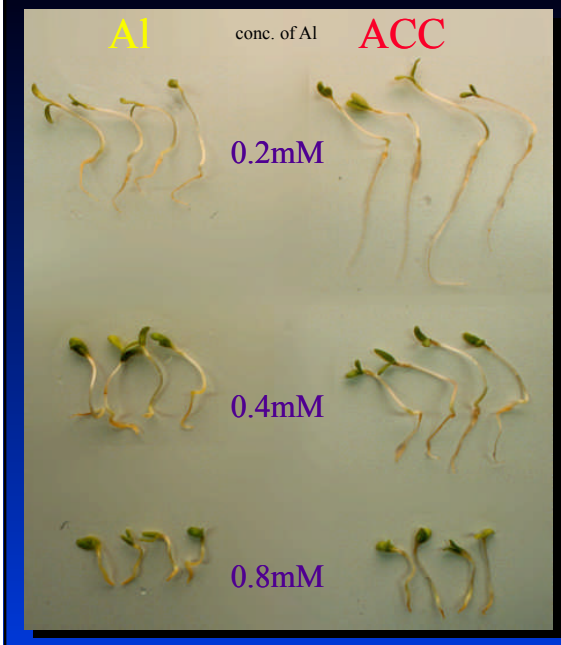
Dimeric AQs in soil are dimerization of monomeric AQs.


The outline of the study in this chapter
CLN
chelating capability
soil profile
CLN contents has a significant relationship with exchangeable Al


Preparation of ACC
0.02mM CLN
(in hot CHCl3)
80ml
0.2mM Al(NO3)3
(in MeOH)
80ml
refluxed
at 60° C
10min
20min
30min
60min
evaporation to
dryness
bioassay

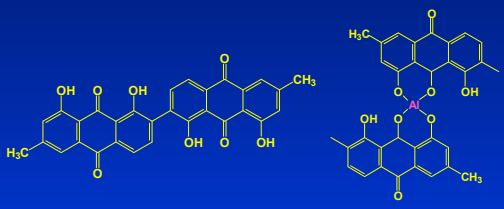

CLN
ACC
absorbance
455nm
535nm
200
400
600
wave length (nm)
UV-VIS absorption spectra of
CLN in CHCl3 and ACC in MeOH
metanolic
HCl
ACC
CLN+Al
solubility
MeOH ○ ×
H2O ○ ×
CHCl3 × ○


Method of bioassay
Al and ACC in 0.1ml MeOH
1
0.5% agar soln. (10ml)
2
Al
ACC
diluted with 0.5% agar soln.
3
mesurement of pH
4
adjust the pH values
dil. HNO3
or
dil. NaOH
lettuce or alfalfa
5
Al medium and
ACC medium
6
incubation in a growth chamber
(for 4days at 20℃ in darkness)

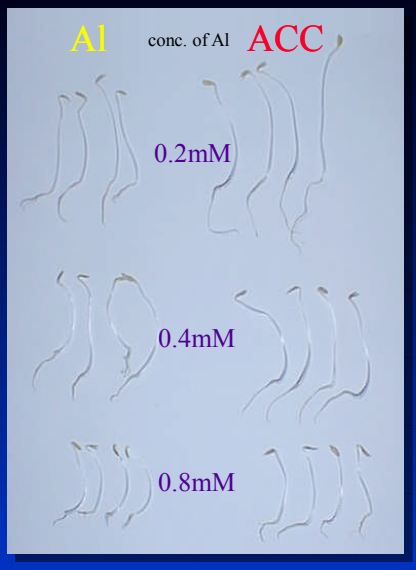


conc. of Al (mM)	root length (cm)		shoot length (cm)	
	Al	ACC	Al	ACC
0.2	2.02	3.26	2.12	2.06
0.4	1.59	1.86	1.91	2.01
0.8	0.68	0.83	1.16	0.99

significant difference (t-test) $p<0.01$
$p<0.05$



The effect of Al and ACC on alfalfa seedlings

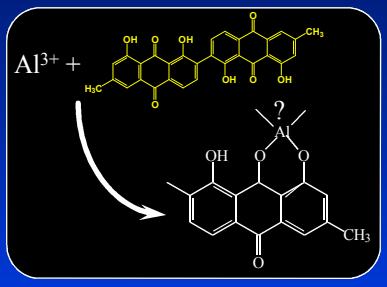


conc. of Al (mM)	root length (cm)		shoot length (cm)	
	Al	ACC	Al	ACC
0.2	1.55	1.74	3.00	3.80
0.4	1.18	1.62	3.00	3.11
0.8	0.52	0.81	2.35	2.29

significant difference (t-test) $p<0.01$

Al^{3+} only

inhibit

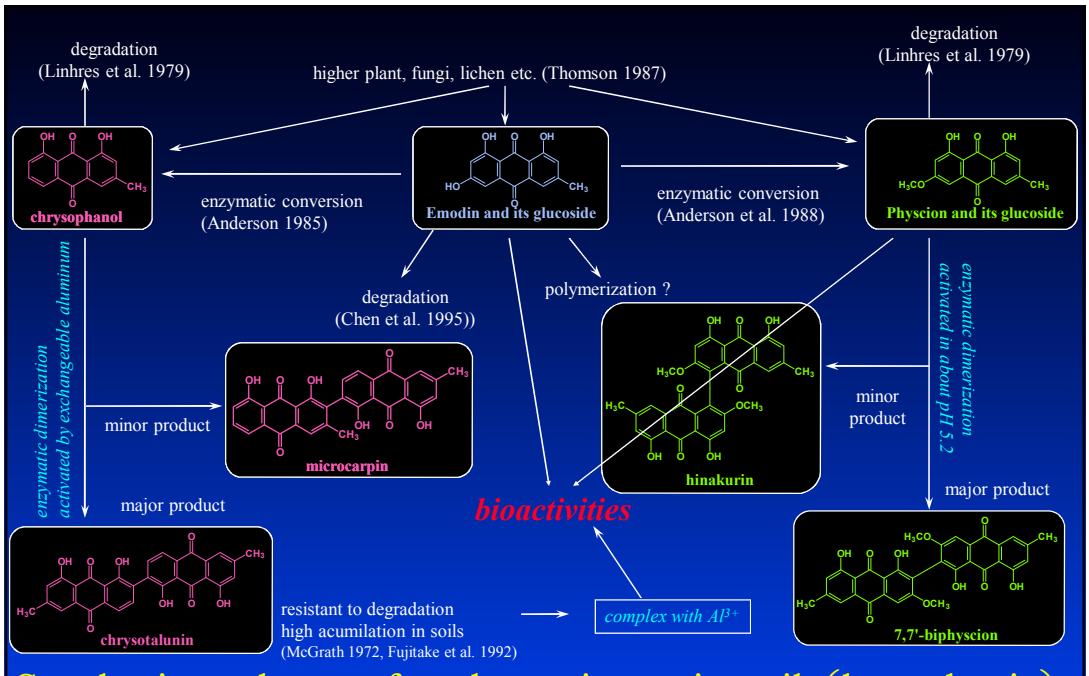


suppress Al toxitity

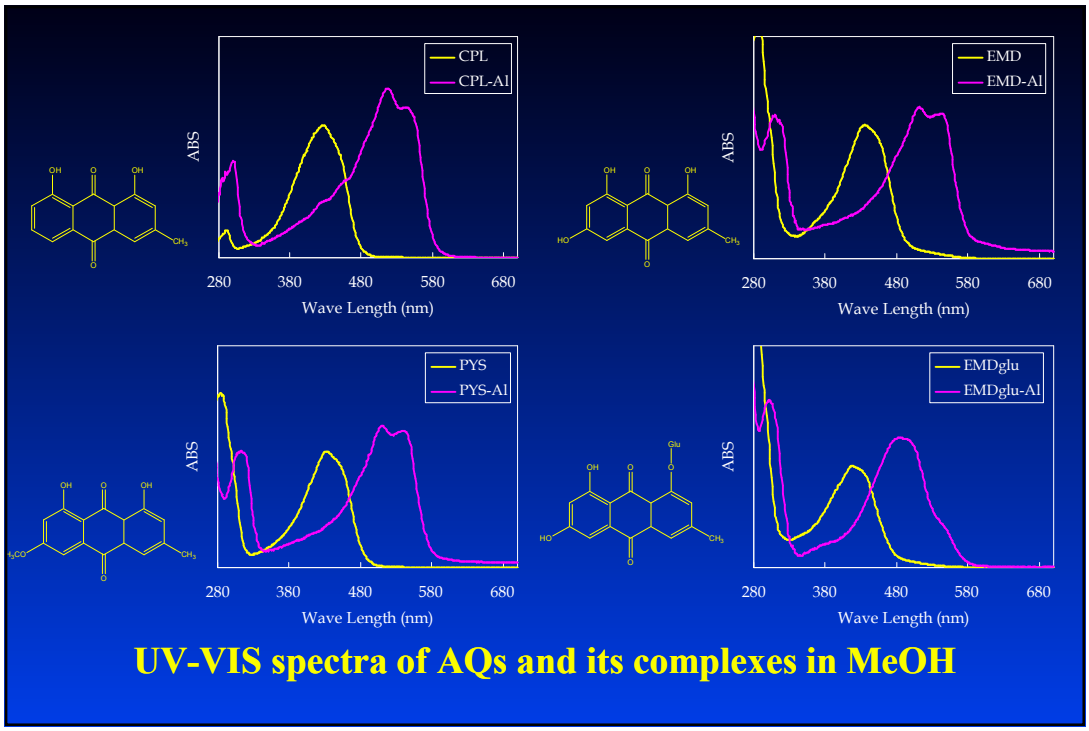
The effect of Al and ACC on lettuce seedlings

Conclusion in this chapter

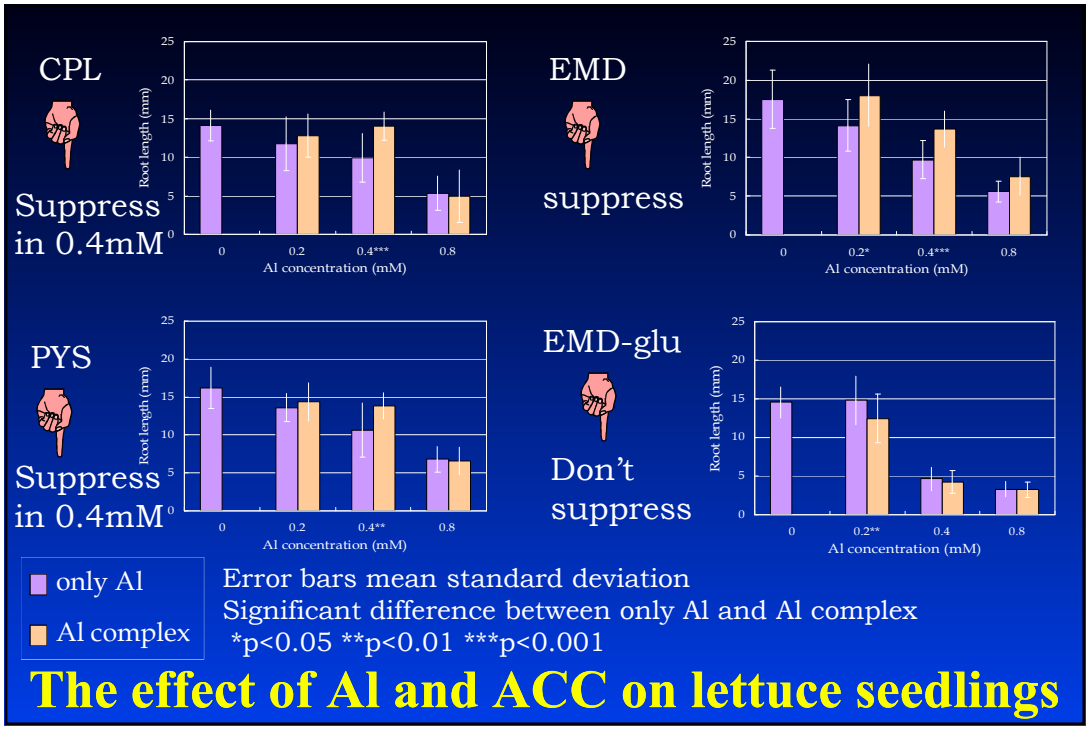
- The main monomeric AQs, and CLN have complex capability with Aluminum
- CLN, PYS, EMD suppress Aluminum toxicity to plant.
- We speculate that CLN contents are correlated to aluminum toxicity



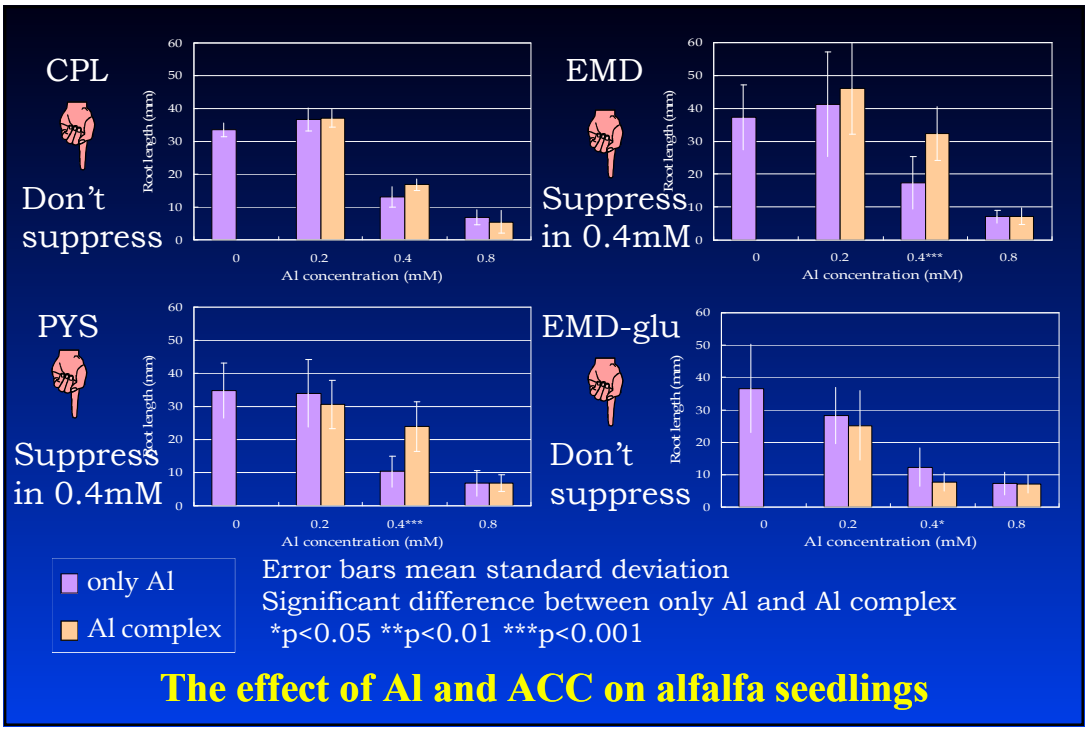
Synthetic pathway of anthraquinone in soil（hypothesis）



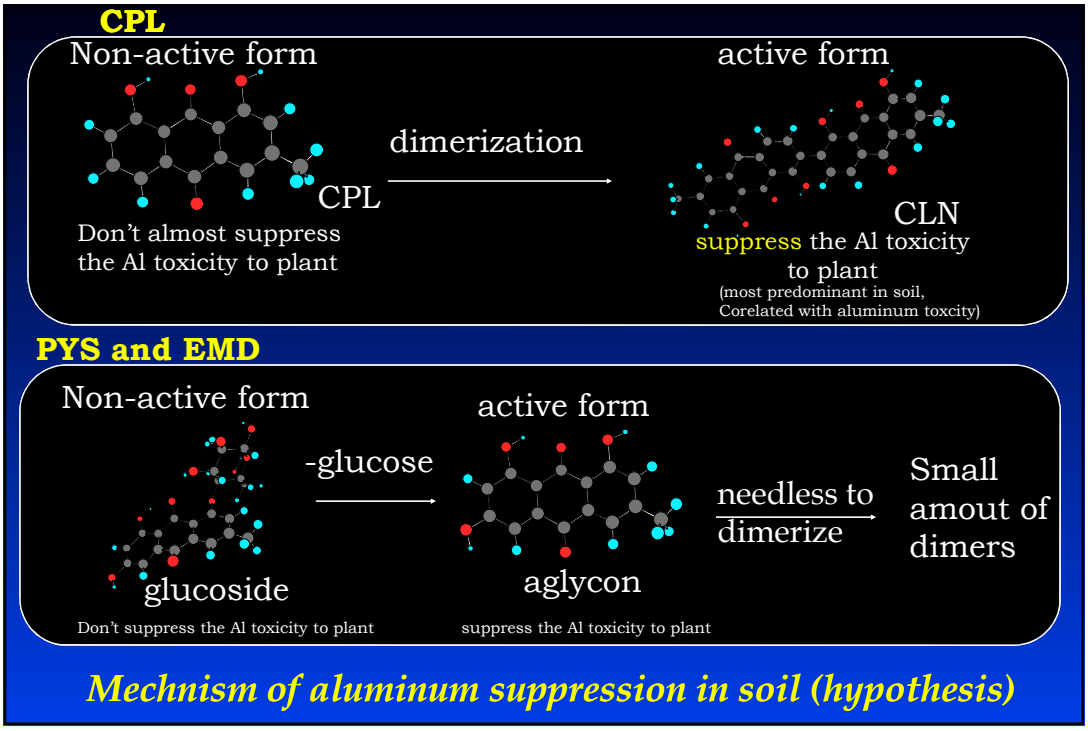
UV-VIS spectra of AQs and its complexes in MeOH



The effect of Al and ACC on lettuce seedlings



The effect of Al and ACC on alfalfa seedlings



Mechnism of aluminum suppression in soil (hypothesis)