anti-snake venom from Cabeca de negra

aq. alcoholic extract "especifico pessoa" of root, 135 ml

cabenegrin A-I, 44 mg 
cabenegrin A-II, 1 mg

antidote against Fer de lance (Bothrops atrox) snake venom

injection of 2.5 mg / kg venom (lethal dose) into 9 kg beagle dog
leads to hypotension, respiratory and cardiac arrest.

injection of 1.0 mg / kg A-I, 15 min before or after venom injection
restores or reverses all effects in 90 min

Effect of A-I on respiratory and cardiovascular responses to snake Venom (Bathrops atorax) in anaesthetized dogs
Ginkgo biloba
Division Ginkgophyta
Order Ginkgoales
Family Ginkgoaceae

ginkgolides

ginkgolide C
**Ginkgo biloba, the ginkgo tree**

One of the first tree, *Ginkgoales*, appeared 250 million years ago.

All species vanished, except one, *Ginkgo biloba*, which remains unchanged 150 million years.

Mentioned in Chinese materia medica, 2800 B.C.  
Inhibitor of platelet activating factor.  
$3$ billion of *Ginkgo* tree extract sold over-the-counter annually.
Summary of ginkgolide isolation from *Bioginkgo* extract

1. Solid-liquid Extraction
2. Bilobalide separation by chromatography
3. Ginkgolide functionalization (benzylation, etc.)
4. Separation by chromatography
5. De-functionalization (debenzylation, etc.)

1-2 days


Syntheses of 7-[³H]-GB & 7-[¹⁸F]fluoro-GB

¹⁸F: positron emitter, $T_{1/2} = 110$ min. produced from $\text{H}_2^{18}\text{O}$ in in-house cyclotron.

Goal: to visualize ginkgolide action sites in living brain by PET (Positron Emission Tomography).


Goal: to study GB and its action site interactions by in vivo autoradiography and in vitro competitive binding studies.

Suehiro, Simpson, Underwood, Castrillon, Nakanishi, van Heertum, Planta Medica, 2005, 71, 622

hippocampus: site of long term potentiation (LTP)
Biotinylated derivs. for photo-crosslinking

**Symbiotic**

Amphiprion clarkii

2 - 3 cm long

60-90 days old

**Non-Symbiotic**

Radianthus kuenkenthali

**Related Text**

- Amphiprion clarkii: 2 - 3 cm long, 60-90 days old
Amphikuemine, aymbiosis inducing substance

Secreted by the sea anemone Radianthus kukenthali
Specifically attracts the anemone fish Amphiprion perideraion.

15 kg of sea anemone homogenate

1 % acetic acid / 20 % aq. MeOH

extract

residue

0.048 mg of symbiont (corresponding to 70% yield)

Structure; Murata, Naya...

Synthesis; konno, Naya (1990)

Konno, Qin, Nakanishi, Murata, Naya, *Heterocycles*, 30, 247 (1990)
Shark Repellents from Flatfish

Pardachirus marmoratus, "Moses sole" (Red Sea) : Clark (1974)
mixture of detergent-like compounds very difficult to separate
also present in P. pavoninus (Indian Ocean, Ryukyus)

I) Steroidal saponins


rings A/B cis and trans

\[
\text{pavoninins} : \text{glcNAc at C-7 or C-15} \\
\text{mosesins} : \text{gal or gal-6-Ac at C-7}
\]

Tachibana, Gruber (1985~88)
Gargiulo (1989) synthesis

II) Amphiphilic 33-peptides

similar to bee venom "mellitin"

Tachibana, Thompson, Kubota (1986~89)

Brevetoxins

red-tide toxins produced by dinoflagellate
massive fish-kils, mollusk poisoning

brevetoxin-B
Lin et al. (1981)
biosynth.: Lee et al. (1986)
Chou et al. (1987)

brevetoxin-A
Shimizu et al. (1986)
Pawlak et al. (1987)
Philanthus wasp

Honey bee prey

Chemical structure: [formula image]
Spider toxins

Argiopine
Argiotoxin 636
Grishin et al. 1986
Adams et al. 1987

JSTX-3
Aramaki et al. 1986

Wasp toxins
Philanthotoxin-433
Eldefrawi et al. 1988
Piek et al. 1988
Age-Related Macular Degeneration (AMD)

- Leading cause of blindness in people over 60
- No cure or treatment
- Progression of atrophic (dry) AMD
- RPE cell atrophy leads to photoreceptor cell death and loss of vision

RPE: retinal pigment epithelium
- Critical for vision since it stores and supplies nutrients, e.g., retinol
- Phagocytoses used outer segment disks

Age-Related Macular Degeneration (AMD)

* Macular degeneration is damage or breakdown of macula
* Close work becomes difficult or impossible
* Many elderly people develop AMD as part of natural aging process, eventually leading to untreatable blindness.
* AMD affects >10 M in U.S.A., and ca. one in every three people over the age of 65.
* There is no remedy for this disease.

**Symptoms associated with AMD**

A dark or empty area in the center of sight
Straight lines distorted
Words look blurred

(from Am. Acad. of Ophthalmology "Keeping an eye on your sight.")
**A2E Fluorophore**

Human RPE cells  
(from > 250 eyes, > 40 yrs. old)

Orange fluorophore < 100 µg

all-trans retinal (1 g),  
ethanolamine, pH 5.2 with AcOH

dark, rt  
20 min

5 mg

revised  
geometrically rigid wedge shape

structure: N. Sakai et al. JACS 118, 1559 (1996)  
synth.: R. Ren et al. JACS 119, 3619 (1997)
A2E FAB-MS w/wo irr. blue light, 450 nm, 10 min, PBS

Common name: bilberry, blueberry
Northern Europe, western Asia, and western North America
Edible fruits with history of medicinal usage
Clinical application
  - Ophthalmologic disorders
  - Vascular disorders
  - Diabetes mellitus

Bilberry extract vs. blue-light induced A2E epoxidation

- A2E in PBS; Tungsten halogen source (470±20nm; 0.4mW/mm²); 8 min exposure
- FAB-MS (3-Nitrobenzyl alcohol as matrix)

Anthocyanins from bilberry extract

Young Jang et al., Photochem. Photobiol. 81, 531 (2005)
Antibody generation against two polyene moieties of A2E

Abeywickrama et al., PNAS, 2007, 104, 14610-14615
**retinal / vitamin A cycle**

- **11-cis-retinal** (less stable)
- **trans-retinal** (more stable)
- **rhodopsin**
- **carotenoids**
- **11-cis-retinol dehydrogenase** (cellular retinal BP)
- **all-trans-retinol dehydrogenase**
- **trans-retinol**
- **lecithin retinol acyl transferase (LRAT)**
- **trans-retinyl esters** (R: primarily palmitate)

- **11-cis-retinol ester** hydrolysis: 5 kcal gain
- **4 kcal uphill** hydrolysis, isomerization

**(Bob Rando)**
Selective capture of retinoid binding proteins

retinal pigment epithelium membrane

Boc-Lys tether

biotinylated trans-retinyl chloroacetate
[Nesnas, Rando, Nakanishi, Tetrahedron, 2002, 58, 6577]

 labeling blocked by trans-retinyl bromoacetate but not by oleyl acetate

(i) incubation, 12 h, rt
(ii) dialysis
(iii) Neutravidin beads, 3 h, 4°C

beads

cross-linked moiety

Selective capture of proteins from bovine RPE

selective capture of only few proteins from a mixture of perhaps thousands

25 kDa LRAT: lecithin retinal acyltransferase

31 kDa RGR: retinal G protein coupled R (11-cis-retinol dehydrogenase)

63 kDa RBP

Rh / G protein cross-linking

peptide 1 (2eq)

ROS (1eq)

\[
\text{irr. 100 W, 3 min, 0°C} \quad \text{irr. 254 nm, 15 min, -178°C}
\]

(meta II)

\[
\begin{align*}
\text{CNBr cleavage, acidic conditions} \\
\text{Click Chemistry}
\end{align*}
\]

photolabeled Gt\(_y\)(60-71) for peptide 3

peptide

Mixture

tandem M.S.

Binding site

HPLC

Fluorescence detection

Mixture

(Y-H. Chen)
Does bee vision differentiate two enantiomers of flower pigment?

olfactory  
(S)-Limonene (R)-Limonene  
turpentine orange

taste  
L-glutamate D-glutamate  
*umami* (the fifth taste sensation)
tasteless

Can vision recognize chirality?

Light vs. Rodopsin

Retinal is in chiral environment, thus CD active

Fishkin, Berov a, Nakanishi  
Flavonol Glycoside Pigments are responsible for Ultraviolet Absorption in Nectar Guide of Flower

*Rudbeckia hirta* (black-eyed susan)

Fig. 1. (A) *Rudbeckia hirta*, photographed in visible light. (B) Same, photographed with ultraviolet transmitting lens and filter, in ultraviolet light. The absorbent basal portions of the petals are the “nectar guides.”

*Science* 1972, 177, 528-530.

*PNAS* 2001, 24, 13745-13750.
Can vision recognize chirality?
-Biological Assays using bees-

Quercetin D-glucoside

Quercetin L-glucoside

Quercetin D-glucuronide

Quercetin L-glucuronide

Tohru Taniguchi
Loose Jaw Fish and Photosensitizers

- 545 nm Rhodopsin
- Chlorophyll derivatives as sensitizers
- Bleaching at 671 nm faster than at 554 nm
Emits 700 nm Light
But Only Contains a 545 nm Rhodopsin

Typical Experiment
Studying Nature and learning from Nature

* Nature is efficient and sophisticated

* Intriguing problems lie in borderline areas

* Interdisciplinary approach is essential

* Such approaches have become feasible only recently

* As questions are answered, further enigmas emerge

* Broad and imaginative thinking is essential