BALSAMS, RESINS, TERPENOPHENOLOID AND PHLOROGLUCINE DERIVATIVES

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1. MACROMORPHOLOGICAL EVALUATION

Benzoe tonkinensis Benzoin, Siam
Styracaceae

Styrax tonkinensis (Pierre) Craib
Styrax benzoides Craib
Styrax benzoin Dryand

Ph.Hg.VIII., Ph.Eur.

It consists of flat or sometimes spherical light or dark brown pieces. In the basic of substance whitish granules are visible, their colour turns light brown during storage. It breaks easily with a conchoidal surface and has vanilla odour.
Its taste is slightly acrid, aromatic.

Balsamum peruvianum Peru Balsam
Myroxylon balsamum (L.) Harms Fabaceae
M. pereirae (Royle) Harms

Ph.Hg.VIII. Ph.Eur.

It is a dark red-brown, slightly viscid liquid, transparent in a thin layer, having a characteristic odour reminding somewhat of vanilla and a permanetly bitter, acrid, burning taste. It neither sticks nor dries up in the air. Crystals do not get separated even after prolonged standing.

Cannabis indicae folium Indian hemp leaf
Cannabis sativa L. var. indica Cannabinaceae

Narcotic

The morphology of the leaves of this tall dioecious herb varies as a function of their insertion point: at the base of the stalk, they are opposite and digitate with five to seven folioles, whereas near the apex of the stalk they are alternate, and either uni- or trifoliolate; the folioles are lanceolate and dentate. The staminate flowers are grouped in panicles, and the pistillate flowers are thinly gathered into compact cymes mixed with foliaceous bracts.

Fiber Variety, Resin Variety

Although it had been thought for a long time that the *C. sativa* species comprised at least two varieties, it is now known that in fact hemp adapts to almost all ecological conditions. Three types of hemp are distinguished, based on the concentrations of

tetrahydrocannabinol (pharmacologically active Δ^9 – THC, commonly referred to as THC), and in *cannabidiol* (CBD, inactive, but a good identification marker):

- the „drug" (resin) type with high THC concentracion (<1%) and no CBD; this type of composition is observed in all of the hemp growing in warm climates, and producing abundant resin;
- the „hemp" (fiber) type with very low THC concentration (<0,3%) the „textile" varieties cultivated in northern temperate climates) and high CBD concentration;
- the „intermediate" type, with high concentrations of both compouds THC and CBD.

Lupuli flos Hop strobile
Humulus lupulus L. Cannabinaceae

Free in trade

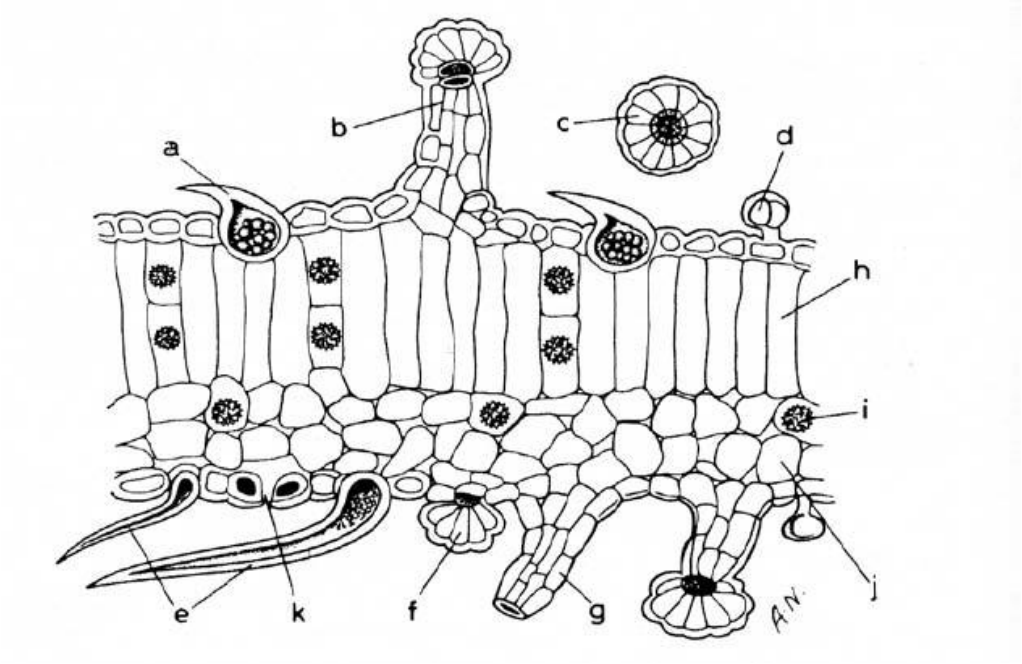
Hop is a tall dioecious perennial herb, with tri- to pentalobate leaves, with pistillate flowers gathered in racemes commonly referred to as hops, hop cones, or strobiles.

The hop cone, greenish-yellow, ovoid, is formed of memranous scales (bract and bracteoles), which partially overlap, and have fine veins. Bract and bracteoles have oleoresin glands they appear as small orangy red granules, and contain lupulin.

2. MICROSCOPICAL TESTS

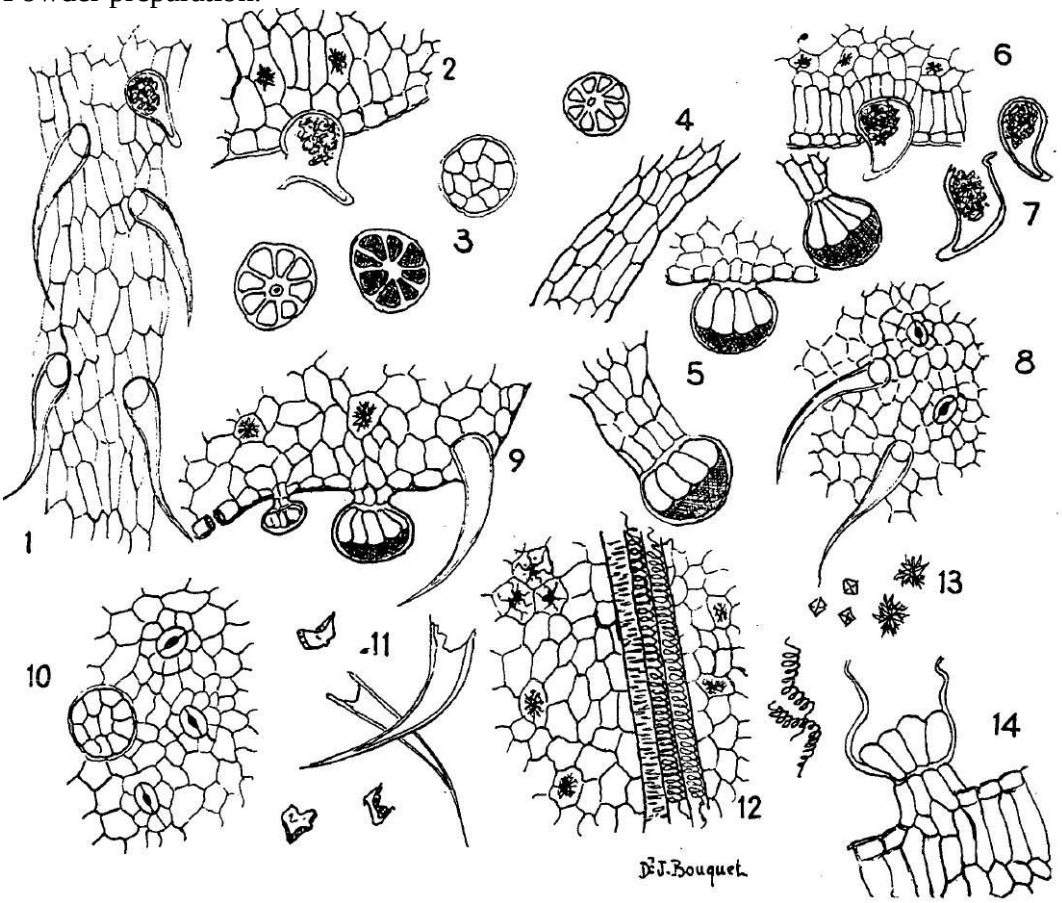
The resin is secreted by numerous hairs. The head is usually 8-celled and the pedicle multiseriate or unicellular. A new type of sessile gland is also described. Abundant conical, curved, unicellular hairs are also found, many having cystoliths of calcium carbonate in their enlarged bases.

Cannabis indicae folium



a: cystolithic trichome;
b: large glandular trichome with several cells in head and stalk;
c: head of one of the large glandular trichomes;
d: small glandular trichome with bicellular head and unicellular stalk;
e: thick walled conical trichomes;
f: large developing glandular trichome;
g: stalk of a large glandular trichome;
h: palisade parenchyma;
i: cluster crystal;
j: spongy parenchyma;
k: stoma

Powder preparation:



Legend

1. Fragment of bract with pointed unicellular covering hairs and hairs with cystoliths of calcium carbonate.
2. Fragment of epidermis with a broken cystolithic hair and twin crystals of calcium oxalate.
3. Four resin-secreting hairs seen from the front: one is still swollen with resin, two are empty and the fourth (beside figure 3) still has its cuticle.
4. Fragment of pedicel of a secretory hair.
5. Three secretory hairs: two pedicellate, one sessile: the oleo-resin is swelling and dilating the cuticle.
6. Fragment of upper epidermis of leafstalk with cystolithic hair.
7. Two detached cystolithic hairs: one intact, the other broken.
8. Fragment of lower epidermis of leaf or bract with two covering hairs and two reniform stomata.
9. Fragment of fruit bract with a covering hair and two secretory hairs in different stages of development; two twin crystals of calcium oxalate in the parenchyma; stoma on left extremity.
10. Fragment of lower epidermis of inflorescent bract: three reni-form stomata, one young sessile secretory hair with cuticle (seen from the front).
11. Two broken unicellular covering hairs; three small pieces of solidified resin.
12. Fragment of floral peduncle with two spiral vessels and one pitted vessel; twin crystals of calcium oxalate and a group of three sclerified cells. On the right, broken spiral vessels such as are frequently found in the preparations.
13. Isolated and twin crystals of calcium oxalate.
14. A pedicellate secretory hair; the cuticle is broken and has released its oleoresin

3. PHYSICAL-CHEMICAL AND CHEMICAL QUALITATIVE INVESTIGATION

3.1. Balsams and resins

3.1.1. Benzoe (Bensoin Siam)
Identification-detection of benzoic acid

Heat a little piece of Benzoe resin (Siam) in dry test tube. First an agreeable vanilla odour and then acrid vapours are evolved from which needle-shaped benzoic acid crystals are deposited in the upper cooler part of the test tube.

Tests of inpurities

- Detection of resin: the pH of the extract of crude- drug with 90% ethanol (1+9) is acidic. Mix water to the solution and the transparent solution turns milkish.
- Detection of free cinnamic acid (exclusion of Summatra Benzoin impurities).

  Heat 0,5 g of a finely powdered sample with 20 ml of 0,1 M potassium permanganate solution; odour of benzaldeyde must not evolve even after prolonged standing.

- Differentiation of Bensoin Siam and Summatra Benzoin: dissolve 0.2 g powdered crude drug in 10 ml 96% ethanol shaking vigorously, filter on cotton. Add 0.5 ml 5% ethanolic $FeCl_3$ solution to 5 ml of the filtrate. Bensoin Siam: green colour (doesn't contain cinnamic acid); Summatra Benzoin: yellow (contain cinnamic acid)

3.1.2. Balsamum peruvianum
Identification – detection of cinnamic acid

*Solubility:*insoluble in water; soluble in alcohol, chloroform, cc. acetic acid. Partially soiluble in ether.

pH: the pH of the solution of crude drug (shaked with reshly boiled and cooled water (1:9)) is mildly acidic

Cinnamic acid: Boil 1 g of crude drug shaking with 20 ml of water for 2 minutes. Filter the hot reaction mixture through a moistened filter paper. Decant the water from the crystalline precipitate after 2 hours. Add to the precipitate 10 ml of 0,03 M potassium permanganate solution Boil again the reaction mixture; it smells from benzaldehyde.

3.1.3. Detection of aromatic volatile components by TLC

Benzoe tonkinensis
Balsamum peruvianum

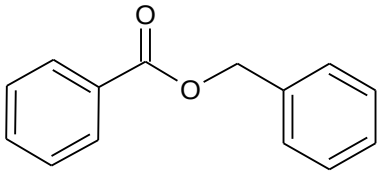
Shake 0,1-0,1 g of the crude drugs with 10-10 ml of ethyl acetate ad use 6-8 µl of the solutions for TLC beside reference solutions of benzaldehyde and vanillin (20 mg/10 ml of ethylacetate).

TLC parameters

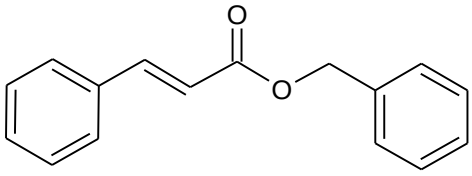
Adsorbent: Kieselgel 60 F_{254}, 0,2 mm
Developing system: petroleum ether-diethylether (7+3)
Evaluation: 1. in UV-254 light
2. Reagent: A: DNPH (dinitrophenyl-hydrazin)/R-sulphuric acid
B: phosphomolibdenic acid (80°C, 5 min)

Reference: 1. benzaldehyde (20 mg in 10 ml etylacetate): 5µl
2. vanillin (20 mg in 10 ml etylacetate): 8 µl

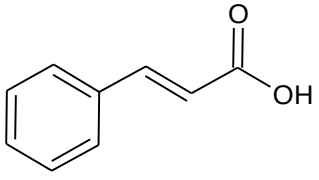
Components	**R_f value**	**R_f value (relative to vanillin)**
benzyl-benzoate	0,80	3,30
benzyl-cinnamate	0,75	3,0
cinamic acid	0,10	0,40



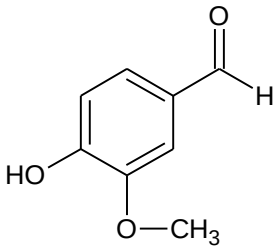
benzyl-benzoate



benzyl-cinnamate



cynnamic acid



vanillin

3.2. Cannabinoids - terpenophenoloid derivatives

3.2.1. Distinction of hashish (resin) and fiber types of hemps by TLC

Preparing of sampes

Shake 0,3 g of powdered drug with 15 ml of petroleum ether in ultrasonic bath for 5 min, then filter the extracts and evaporate it to 1,0 ml. USE 2-5 µl of the solution for TLC beside 2 µl of reference substance: Δ^9THC solution (0,02% in $CHCl_3$).

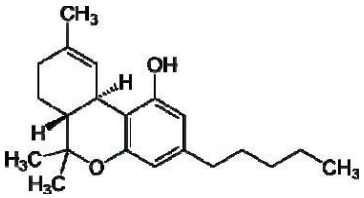
TLC parameters

Adsorbent: Kieselgel 60 F_{254}, 0,2 mm
Developing system: n- hexane-dioxane (4+1)
Reference: Δ^9THC: 5µl
*Reagent for spray: 0,*2% of Echtblausaltz B (di-o-anizidine-tetrazolium-chloride) in 96% ethanol
Evaluation of chromatogram: more colouring spots are visible: according increasing of R_f values

Activity of cannabinoid compounds:

CBD cannabidiol (antibiotic effect)
CBDA cannabidiol acid (sedative and antibiotic effect)
CBN cannabinol (inactive)
CBNA cannabinol acid (inactive)
CBG cannabigerol (antibiotic effect)
CBGA cannabigerol acid (antibiotic effect)
CBC cannabichromene (sedative)
CBCA cannabichromene acid (sedative)

Δ^9THC tetrahydrocannabinol (narcotic, analgetic)
Δ^8THC tetrahydrocannabinol acid (inactiv)
Δ^9THCA tetrahydrocannabinol (narcotic, analgetic)
Δ^8THCA tetrahydrocannabinol acid (inactiv)



THC

Remark: THC is detectable on chromatogram of hashish hemp. On the chromatogram of fiber hemp THC is not visible or only in traces.

3.3. Hop acids – phloroglucine derivatives

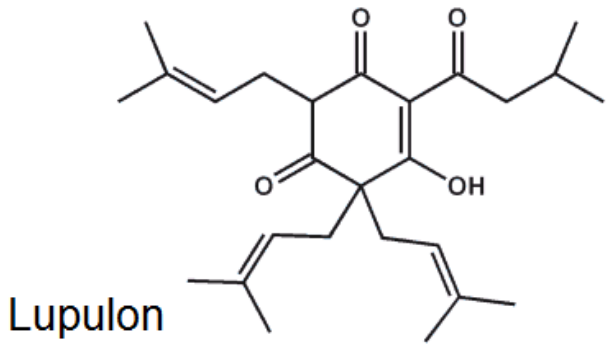
Detection of hop acids in hop strobile by TLC

Preparing of sample

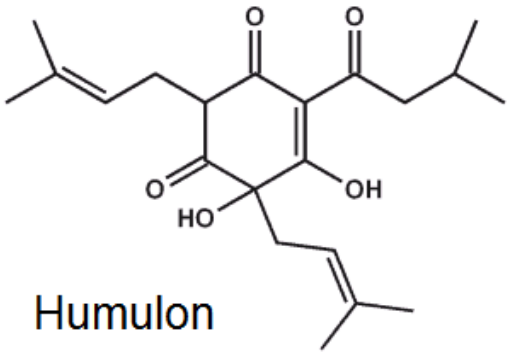
Extract 1 g of powdered drug with 10 ml of methanol-water (7:3) in ultrasonic bath for 5 min and filter. Use 10 µl of the solution and 10 µl of reference solution (1% of humulon and lupulon in methanol).

TLC parameters
Adsorbent. Kieselgel 60 F_{254}, 0,2 mm
Developing system: glacial acetic acid – ethylacetate – cyclohexane (2:38:60)
Reagent for spray: Echtblausaltz B
Reference: sudan-orange (1.0 mg), curcumin (2.0 mg), dimethylaminobenzaldehyde (2.0 mg) in 10 ml of methanol

Evaluation of chromatogram: *1. UV-254, UV-365*
2. spray reagent: Echtblausalz B



Lupulon



Humulon

Report

– TLC aromatic volatile components of balsams
– Distinction of hashish and fiber hemps by TLC of cannabinoids
– TLC of hop acid