Phenolic Constituents of *Galactites tomentosa* (Asteraceae)

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Allergic Contact Reactions, *Galactites tomentosa,* Chlorogenic Acid, 3,5-Dicaffeoylquinic Acid

The Asteracea *Galactites tomentosa*, known as a plant causing allergic contact reactions, was investigated for new constituents in leaves and stems. Chlorogenic acid and 3,5-dicaffeoylquinic acid could be detected by HPLC in tissues and trichome preparations as well.

Introduction

Among the plant families containing species with sensitizing constituents, the Asteraceae is the largest and most important one. *Cynara scolymus* (the artichoke), *Cynara humilis,* and *Centaurea hermanii* which belong to the tribe of Cynareae are well known for its allergy causing properties (Rodriguez *et al.,* 1976; Hausen and Oestmann, 1988; Paßreiter *et al.,* 1988; Ducombs *et al.,* 1990; Ross *et al.,* 1993; Paulsen, 1992, Paulsen *et al.,* 1993).

Another well investigated example is the sun flower (*Helianthus annuus*), which causes allergic contact dermatitis by means of irritating compounds stored in multicellular capitate glandular hairs (Hausen and Spring, 1989).

Sesquiterpene lactones (Hausen and Oestmann, 1988), polyacetylenes (Paulsen, 1992), and phenolic compounds (Ludlum *et al.,* 1991) belong to these constituents, responsible for allergic reactions caused by touching glandular trichomes or prickles.

Comparable reactions (dermatitis) have been reported from the mediterranean thistle *Galactites tomentosa* after harvesting (U. Reifenberger, personal communications).

Constituents of *G. tomentosa* have previously been investigated by Catalano *et al.* (1983) and Christensen and Lam (1990). These authors found several major sugars (rhamnose, fructose, glucose), terpenes and two polyines.

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Objective of this study was to reveal possible mechanisms, causing allergic reactions by reinvestigation of secondary metabolites of *G. tomentosa* and the fine structure of leaves as well.

Experimental

Plant material

The plant material was obtained from the horticulture of the University of Bielefeld. Seeds of *Galactites tomentosa* (Asteraceae) used in these experiments were supplied by U. Reifenberger from the Canary Island Gomera. Plants of *Cynara scolymus* were supplied from a local market.

The plants of *G. tomentosa* were grown under greenhouse conditions on a mixture of 50% (w/w) compost-ground and 50% (w/w) soil type "Fruß-dorfer Einheitserde".

Preparation for HPLC

30g plant material (fresh leaves and stems) of *G. tomentosa* were crushed and extracted with MeOH for 12 hours. After removing the solvent under vacuum, the residue (4 µl) was dissolved in 2 ml MeOH.

Isolation of plant-hair constituents

The hairs of fresh leaves of *G. tomentosa* (30 g) frozen in liquid nitrogen were depilated with a razor-blade.

The depilated hairs (2 mg) were sonicated (3x60 sec., interrupted by 30 sec. pause) in 5 ml MeOH (LABSONIC 1510, 400 Watt) and then extracted over night.

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After filtration the solvent was removed under vacuum and the residue (3 µl) dissolved in 2 ml MeOH and used for HPLC.

Preparative HPLC and UV-spectra

The HPLC was recorded on a HPLC-system from WATERS (W 600, W 717 plus autosampler, W 994 photodiode-array-detector for UV-spectra) with a NUCLEOSIL 120–5 C_{18}-column (ET/250/8/4) from MACHEREY -NAGEL.

Solvent: A: 1.5% H_3P0_4; B: 80% CH_3CN (v/v). Gradient: in 30 min from 0 to 100% B with a flow rate of 1 ml/min.

Two ml of the methanolic extracts of *G. tomentosa* were diluted 1:10 and filtered. The trichome-extracts were not diluted. 20 µl of each extract were used for HPLC.

For comparison with HPLC-data of leaf extracts, a chlorogenic acid-standard was subjected to HPLC-analysis.

Preparation for MS

The solvent was removed from the two compounds, separated form leaves by HPLC, under vacuum and the residues were dissolved in 0.5 ml EtOAc.

MS were run on an AMD-402, AMD Intectra as L-SIMS (**L**iquid **S**econdary **I**on **M**ass **S**pectroscopy). Molecular fragmentation of the samples was achieved by Bombardment with cesium-ions. Matrix: glycerol.

NMR-spectra

The NMR-spectra were taken on a BRUKER ARX 400 at room temperature, locked to deuterium resonance of the solvent CD_3OD.

Scanning electron microscopy (SEM)

Small pieces of leaves were fixed with glutaraldehyde (2.5% v/v in phosphate-buffer, pH 7.2) for 3 h and dehydrated by a graded acetone series (30, 45, 60, 75, 90 and 100% v/v). Dryness was achieved in liquid CO_2 in a critical point dryer (BALZERS UNION). Finally, the plant material was sputtered with gold (33nm with HUMMER VII, ANATECH Ltd) and observed on a HITACHI SEM-450.

Results and Discussion

Searching for irritating compounds such as sesquiterpene lactones in leaves and stems of *Galactites tomentosa*, TLC was applied in preinvestigations to extracts of this plant and another member of the tribe of Cynareae, *Cynara scolymus* (results not shown). This plant is also well known for its allergy causing properties (Rodriguez *et al.* 1976) and probably contains the same irritating compounds as *G. tomentosa*.

Comparison of both species (by TLC) shows distinctly identical constituents (spots with identical color and R_f-values). This indicates that presumably allergic compounds of *Cynara scolymus* are accumulated likewise in *G. tomentosa*. Some of these constituents of *Cynara scolymus* were identified by Bernhard and Thiele (1979) who isolated sesquiterpene lactones such as cynaropicrine, dehydrocynaropicrine and grosheimine. These sesquiterpene lactones which can be grouped together as guajanolids are probably characteristic for all Asteraceae of the Cynareae tribe and may be considered as the allergy causing principle in *C. scolymus* and *G. tomentosa* . In the case of *G. tomentosa* further investigations of plant extracts by GC-MS are necessary for exact compound identification.

Analysis of extracts of leaves and plant hairs of *G. tomentosa* led to the identification of 2 phenolic constituents, namely chlorogenic and 3,5-dicaffeoylquinic acid.

Retention times: chlorogenic acid: 15.08 min; 3,5-dicaffeoylquinic acid: 18.71 min.

Maxima of UV-absorption: chlorogenic acid 324.2 nm, 3,5-dicaffeoylquinic acid 326.6 nm.

MS-data: chlorogenic acid: MS *m/z:* 354 $[M]^+$ (13.1); 336 (75.2); 180 (81.6); 163 (100); 135 (15.6)

3,5-dicaffeoylquinic acid: MS *m/z:* 498 $[M-H_2O]^+$ (2.8); 392 (2.1); 336 (32.6); 180 (68.8); 163 (100); 135 (16.3)

^{1}H-NMR-data

Chlorogenic acid: ^{1}H-NMR (CD_30D) = 7.60 [d, H-7'; J(7'-8') 15.9]; 7.09 [d, H-2'; J(2'-6') 2.0]; 6.99 [dd, H-6', J(5'-6') 8.2]; 6.81 [d, H-5']; 6.31 [d, H-8']; 5.37 [ddd, H-5; J(5–6) 9.1; 4.6; J(4–5) 8.8]; 4.30 [ddd, H-3, J(3–4) 3.1; J(2–3) 3.2; 4.9]; 3.76 [dd, H-4]; 2.30–2.05 [m, H-2, H-6].

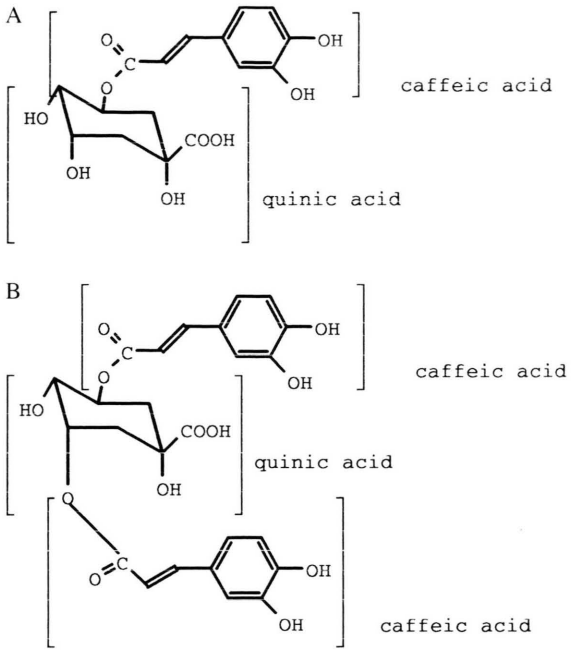

Fig. 1. Structure of **A**: chlorogenic acid; **B**: 3,5-dicaffeoylquinic acid.

3,5-dicaffeoylquinic acid: ^{1}H-NMR (CD_3OD) = 7.66; 7.62 [d x2, H-7' x 2, *J*(7'-8') 16.0], 7.11; 7.10 [d x2, H-2' x2, *J*(2'-6') 2.1], 7.01; 7.01 [d x2, H-6' x2, *J*(5'-6') 8.2]; 6.82 [d x2, H-5']; 6.40; 6.31 [d x2, H-8']; 5.46 [m, H-3, H-5]; 4.00 [dd, H-4; J(3–4) 3.2; *J*(4–5) 7.8]; 2.35 [dd, H-6A, *J*(6A-5) 3.8; *J*(6A-6B) 14.1]; 2.24 [m, H-2A/2B]; 2.19 [dd, H-6B, J(6B-5) 6.6].

Chlorogenic acid was identified by comparing UV-spectroscopic data and retention times of a standard with analytical data of the specimen. The structures of the isolated plant constituents shown in Fig. 1**A** (chlorogenic acid) and 1**B** (3,5-dicaffeoylquinic acid)were deduced on the bases of mass and ^{1}H-NMR-spectra. The structure of 3,5-dicaffeoylquinic acid shows a duplicate number of proton signals, indicating that the quinic acid carries two caffeic acid substituents. The spectroscopic data are equal to those measured by Wald *et al.* (1989). The chemical shifts of the H-3 and H-5 signals indicated connection of the caffeic acid substituents with the C-3 and C-5 of the quinic acid.

The strucutre of the phenolic substance 3,5-dicaffeoylquinic acid is similar to Cynarine, isolated from *C. scolymus* by Panizzi and Scarpati (1954). The presence of those Cynarin-comparable quinic acid esters in *Galactites tomentosa* points out that these constituents may be a characteristic chemotaxonomic feature in the Cynareae tribe but they are not responsible for allergic contact reactions.

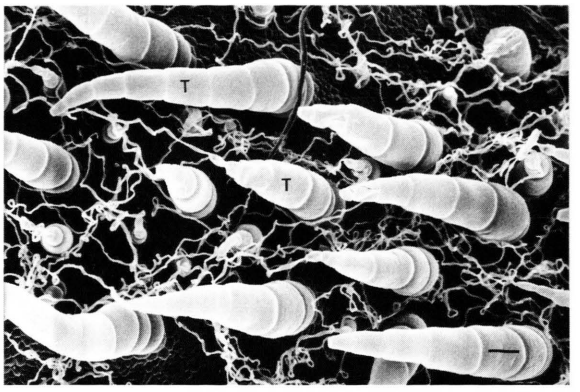

Fig. 2. Hairy upper leaf side of *G. tomentosa* . Bar = 50 µm. T = trichome. The trichomes are multicellular and consist of up to 8 cells.

Ravn *et al.* (1988) classified phenolic substances in plants as phytoalexins, which are usually synthesized in epidermic cells. These compounds show antibacterial activity and therefore seem to protect the plant against infections and also towards other

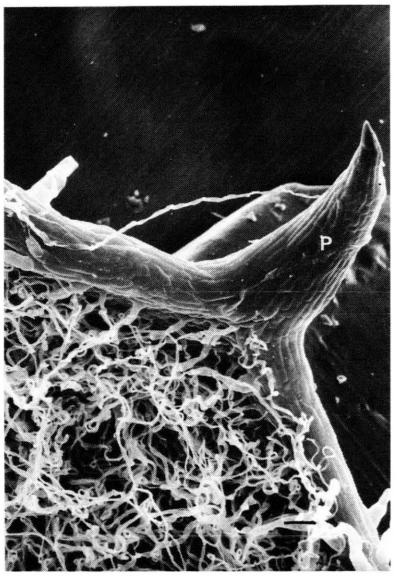

Fig. 3. Hairs on the bottom side of *G. tomentosa* leaves. P = Prickle. Bar = 50µm.

pests. The phenolic compounds identified in *G. tomentosa* obviously are located in its plant-hairs.

To get more knowledge about mechanisms responsible for the release of allergic constituents (e.g. glandular trichomes) of *G. tomentosa,* scanning electron microscopical (SEM) investigations of leaves were carried out.

SEM-observations did not reveal any glandular hairs on the upper leafside, only multicellular septated flagellate hairs as described by Ramayya (1968) (Fig. 2) and long flagellate hairs on the bottom side (Fig. 3) could be detected. Both types of hairs from *Galactites* leaves, are not comparable with the stinging hairs of *Urtica dioica.* No silicification could be found which is necessary for penetration of human skin (Southcott and Haegi 1992). Therefore these trichomes are unable to release allergic substances.

The only structures of *Galactites* leaves which may penetrate human skin are, according to SEM-observations, the prickly thornes (Fig. 3). A similar way of skin penetration is referred from *Robinia pseudoacacia* (Roth *et al.*, 1988) which contains the allergic and cytotoxic compounds robine and phasine in cortical tissue and its prickles.

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Bernhard, H.O. and K. Thiele (1979), Cynaratriol, ein neues Guajanolid aus der Kardone *Cynara cardunculus* L. und der Artischocke *C. scolymus* L. (Compositae). Helv. Chim. Acta **62**, 1288–1297.

Catalano, S., M. Pacchiani, E. Tascini and I. Morelli (1983), Constituents of *Galactites tomentosa*. Fitoterapia **54**, 33–34.

Christensen, L.P. and J. Lam (1990), Acetylenes and related compounds in Cynareae. Phytochemistry **29**, 2753–2785.

Ducombs, G., C. Benezra, P. Talaga, K.E. Anderson, D. Burrows, J.G. Camarasa, A. Dooms-Goossens, P.J. Frosch, J.M. Lachapelle and T. Menne (1990), Patch testing with the "sesquiterpene lactone mix": a marker for contact allergy to Compositae and other sesquiterpene-lactone-containing plants. A multicentre study of the EECDRG. Contact Dermatitis **22**, 249–55.

Hausen, B.M. and G. Oestmann (1988), Untersuchungen über die Häufigkeit berufsbedingter allergischer Hauterkrankungen auf einem Blumengroßmarkt. Dermatosen Beruf Umwelt **36**, 117–124.

Hausen, B.M. and O. Spring (1989), Sunflower allergy. On the constituents of the trichomes of *Helianthus annuus* L. Contact Dermatitis **20**, 326–334.

Ludlum, C.T., G.W. Felton and S.S. Duffey (1991), Plant defenses: Chlorogenic acid and polyphenol oxidase enhance toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to *Heliotis zea*. J. Chem. Ecol. **17**, 217–233.

Panizzi, L. and M.L. Scarpati (1954), Constitution of Cynarine, the active principle of the Artichoke. Nature **174**, 1062.

Paßreiter, C.M., M. Florack, G. Willuhn and G. Goerz (1988), Allergische Kontaktdermatitis auf Asteraceae. Identifizierung eines 8,9-Epoxythymoldiesters als Kontaktallergen von *Arnica sachalinensis*. Dermatosen Beruf Umwelt **36**, 79–82.

Paulsen, E. (1992), Compositae dermatitis: a survey. Contact Dermatitis **26**, 76–86.

Paulsen, E., K.E. Andersen and B.M. Hausen (1993), Compositae dermatitis in a Danish dermatology department in one year. Results of routine patch testing with the sesquiterpene lactone mix supplemented with aimed patch testing with extracts and sesquiterpene lactones of Compositae plants. Contact Dermatitis **29**, 6–10.

Ramayya, N. (1962), Studies on the trichomes of some compositae I.: General structure. Bull. Bot. Sur. In. **4**, 177–188.

Ravn, H. and L. Brimer (1988), Structure and antibacterial activity of Plantanajoside. Phytochemistry **27**, 3433–3437.

Rodriguez, E., G.H.N. Towers and J.C. Mitchell (1976), Biological activities of sesquiterpene lactones. Phytochemistry **15**, 1573–1580.

Roth, L., M. Daunderer and K. Kormann (1988), Giftpflanzen-Pflanzengifte. Vorkommen, Wirkung, Therapie – Allergische und phototoxische Reaktionen. 3. Auflage, Ecomed Verlagsgesellschaft. Landsberg, München.

Southcott, R.V. and L.A.R. Haegi (1992), Plant hair dermatitis. Med. J. Aust. **156**, 623–632.

Wald, B., V. Wray, R. Galensa and K. Herrmann (1989), Malonated flavonol glycosides and 3,5-dicaffeoylquinic acid from pears. Phytochemistry **28**, 663–664.