

It should be pointed out that similar results were obtained with congenital and dermal nevi.

In the case of superficial spreading melanomas (SSM), cryo-sections have also been investigated by both the fluorescence method and the histological examination of the HE-stained sections. As in in-situ measurements, there is hardly any fluorescence in the tumor region including the surface of the epithelium (Fig. 3). However, the apparently healthy connective tissue adjacent to the tumor exhibits a high fluorescence intensity. This can be seen even better in Fig. 4 a which agrees very well with in-situ measurements according to which the tissue adjacent to the tumor exhibits the highest fluorescence intensity (up to about 10 times larger than in healthy tissue). It should be pointed out that this is almost the edge of the tumor which spreads towards the left. Furthermore, the bright spot at the epithelium is an artefact. Also in this case, the histological findings (Fig. 4 b) show a similar tumor dis-

tribution like the fluorescence picture shown in Fig. 4 a. Another section of the tumor-affected connective tissue region is shown in Fig. 4 c.

It is interesting to note in Fig. 4 a, c that large sections of the tumor exhibit a brown color which might be identical to that obtained occasionally in the tumor region in in-situ measurements at 535 nm.

From the results obtained with unstained and HE-stained cryo-microsections it can be concluded that the fluorescence pictures of the unstained cryosections not only confirm the histological observations but also reveal additional information about molecular changes. For elucidating these changes in some more detail it seems to be important to determine the type of fiber to which NADH, presumably the fluorophore responsible for the blue fluorescence light with a maximum at about 475 nm, is attached.

Similar results were obtained, e.g., with tissue samples from kidney by using a freeze-drying fixation technique [4].

Since the fluorescence technique used is non-invasive, non-destructive, fast, and is based on naturally occurring fluorophore(s), it might be used in addition to the established methods.

The excellent technical assistance of H. Führer and G. Thiele is appreciated. Part of the work was supported by the Bundesministerium für Forschung und Technologie, the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and from the Carl Zeiss Co., Hoechst, and Ernst Leitz Co., Wetzlar and Frankfurt.

Received March 15, 1989

1. Lohmann, W., Paul, E.: *Naturwissenschaften* 75, 201 (1988)
2. Lohmann, W., Mußmann, J., Lohmann, C., Künzel, W.: *Eur. J. Obstet. Gynecol. Reprod. Biol.* 31, 249 (1989)
3. Lohmann, W., Mußmann, J., Lohmann, C., Künzel, W.: *Naturwissenschaften* 76, 125 (1989)
4. Sjöstrand, F.: *Acta Anat. Suppl.* 1 (1944)

The Synthesis of Caffeic Acid Esters, a New Group of Naturally Occurring Contact Allergens

H.-T. Stüwe, G. Bruhn, and W. A. König

Abteilung Organische Chemie der Universität, D-2000 Hamburg

B. M. Hausen

Experimentelle Allergologie der Universitäts-Hautklinik, D-2000 Hamburg

The current "back to nature" trend confronts us with a continuous increase of cases of allergic contact dermatitis due to natural products. One of the "hits" in the series of materials from natural sources is the bee product, propolis, used preferably in "bio-cosmetics" or "natural cosmetics". Propolis is a yellow-brown to dark brown wax-like odorous material produced by the bees as a glue to seal hive walls and strengthen the borders of

their combs. To prepare this material the bees collect the oily or hardened resinous exudate from the buds of the black poplar, *Populus nigra* L., and mix it with bee wax. In particular the bees embalm dead invaders and other particles found in their comb to prevent putrescence and microbial decomposition. Thus propolis is said to possess antiseptic, antimycotic, spasmolytic, antiinflammatory, and a number of other beneficial properties which sup-

port its current usages. On the skin, for example, propolis is said to develop a smoothing effect.

Since the beginning of propolis usage adverse effects like severe allergic reactions of the skin have been observed. Hundreds of cases of specific hypersensitivity (allergy of the cell-mediated type) have been described [1]. Recently the responsible contact allergens have been isolated and identified. They were all found to be different esters of caffeic acid [2, 3]. Structure elucidation revealed 1,1-dimethylallyl caffeate (3-methyl-2-butenyl-caffeic acid ester) (Fig. 1) to be the main component. As minor constituents 2-methyl-2-butenyl caffeate, 3-methyl-3-butenyl caffeate and phenylethyl caffeate were de-

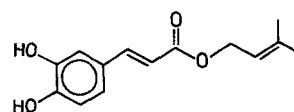


Fig. 1. 1,1-Dimethylallyl-caffeic acid ester

terminated [2, 4]. In guinea pig sensitization experiments as well as by patch tests in sensitized individuals these compounds were shown to be strong sensitizers. Thus it became obvious that all identified caffeates were originally constituents of the poplar buds.

To obtain more material for epicutaneous testing in propolis-sensitive patients and in order to study structure-activity relationships we have begun synthesizing these sensitizing poplar/propolis constituents. In the following we describe the synthesis of the main contact allergen 1,1-dimethylallyl-caffeic acid ester (3-methyl-2-butenyl caffeate).

As far as we know synthesis of these caffeic acid esters has not been done before. Because of the probably highly reactive molecule with two double bonds, it was necessary to use mild reaction conditions for esterification.

One possible way to achieve the unsaturated ester is through the reaction of the cesium salt of commercially available caffeic acid (trans-3,4-dihydroxycinnamic acid) with an alkenyl bromide [5]. The reaction takes place under neutral conditions (pH 7) and at room temperature.

1.8 g (10 mmol) caffeic acid was dissolved in 40 ml methanol. The solution was titrated under stirring to pH 7.0 (pH paper) with a 20 % aqueous solution of Cs_2CO_3 . After 4 h stirring at room temperature, the mixture was evaporated to dryness and reevap-

orated twice with methanol. The light brown residue was dried carefully (oil pump vacuum). Then the caffeic acid cesium salt was suspended in dry DMF and 2 ml (17 mmol) 1-bromo-3-methyl-2-butene were added slowly under ice-cooling and stirring. The course of reaction was studied with thin-layer chromatography (ethyl acetate/petroleum ether 1:1, v:v). After 4 h stirring at room temperature, the mixture was filtered and evaporated in vacuo. The residue was then suspended in ice-water and extracted four times with ethyl acetate. The combined organic solvent was washed with saturated solution of NaHCO_3 , water, 0.1 N HCl, water and then dried over Na_2SO_4 .

The following purification by chromatography (ethyl acetate/petroleum ether 1:1, v:v) gave the pure 1,1-dimethylallyl-caffeic acid ester, yield was 660 mg = 27 %; mp 126 °C.

$^1\text{H-NMR}$ (CDCl_3): δ (ppm) = 1.77 (s, 3H, $-\text{CH}_3$), 1.80 (s, 3H, $-\text{CH}_3$), 4.71 (d, 2H, H_d , $J_{de} = 7.2$ Hz), 5.41 (m, 1H, H_e , $J_{de} = 7.2$ Hz), 6.11 (s, 1H, $-\text{OH}$), 6.27 (d, 1H, H_b , $J_{ab} = 15.8$ Hz), 6.41 (s, 1H, $-\text{OH}$), 6.88 (dd, 1H, 5-H, $J_{56} = 8.3$ Hz; $J_{25} = 0.3$ Hz), 7.0 ($\alpha\alpha$, 1H, 6-H, $J_{65} = 8.3$ Hz, $J_{62} = 2$ Hz), 7.11 (dd, 1H, 2-H, $J_{26} = 2$ Hz, $J_{25} = 0.3$ Hz), 7.60 (d, 1H, H_a , $J_{ab} = 15.8$ Hz).

The direct-inlet mass spectrum (EI) showed the M^+ at $m/e = 248$.

The instability of the molecule was shown by different side reactions. During prolonged storage in solution at

room temperature, the color of the solution changed from clear to yellow-brown.

One explanation for the moderate yield of the esterification is the little solubility of the cesium salt in DMF as a solvent. In a second experiment we tried to improve the yield by choosing abs. methanol as a polar solvent. The cesium salt is fully soluble in methanol, therefore better reaction conditions should be obtained. After the work-up the analytical data of $^1\text{H-NMR}$ spectroscopy and mass spectrometry showed that only the caffeic acid-methyl ester had been isolated. This means, that the forming 1,1-dimethylallylic ester was fully transesterified to the corresponding methyl ester.

Synthesis of the other minor caffeates is in progress.

Received February 10, 1989

1. Hausen, B. M., Wollenweber, E., Senff, H., Post, B.: *Cont. Derm.* 17, 163 (1987)
2. Wollenweber, E., Asakawa, Y., Schillo, D., Lehmann, U., Weigel, H.: *Z. Naturforsch.* 42 c, 1030 (1987)
3. Hausen, B. M., Wollenweber, E., Senff, H., Post, B.: *Cont. Derm.* 17, 171 (1987)
4. Hausen, B. M., Wollenweber, E.: *ibid.* 19, 296 (1988)
5. Wang, S. S., Gisin, B. F., Winter, D. P., Makofske, R., Kuleska, I. D., Tzoug-raki, C., Meienhofer, J.: *J. Org. Chem.* 42, 1286 (1977)

Physiological Variability of Auditory Neurons in a Grasshopper

Comparison of Twin Cells and Mirror-Image Cells

A. Stumpner

Institut für Zoologie II der Universität, D-8520 Erlangen

Invertebrates are well suited for interneuron research, since many of their neurons show a characteristic morphology. This has led to the concept of the "unique, individually identifiable neuron" [1] and has even made possible a comparison across species [2]. How-

ever, comparison of the physiological properties of the members of a morphologically defined neuron type may reveal considerable differences. As an explanation for this "variability" two basic possibilities come into considera-

(1) A set of identically shaped neurons exists (e.g., twins or triplets [3]), which differ in physiology, and different members of this set may have been recorded in different preparations.

(2) There is indeed variability of one unique neuron type between individuals.

In the auditory pathway of *Locusta migratoria* twin neurons have been reported in two cases ([3]: SN1, BSN1). These twins may have different functions in auditory processing, since only a part of the recorded SN1-individuals exerted an inhibitory influence on an identified higher-order interneuron (AN1, see [3]).

In *Chorthippus biguttulus* the homologues of both neuron types, SN1 and