Examination of the Chemical Composition of Propolis I. Isolation and Identification of the 3,4-Dihydroxycinnamic Acid (Caffeic Acid) from Propolis

The chemical composition of the bee's product, propolis, has been examined very little up to now. Among the types of chemical substances found in propolis are waxes, resins, balsams, aromatic and ethereal oils, pollen and other organic matter. The proportion of these types of substances varies and is dependent on the place and time of collecting propolis. Generally it is stated that propolis contains about 30% wax, 55% resins and balsams, 10% ethereal oils and approximately 5% pollen.

Several authors have so far worked on proving the presence of the above-named substance in propolis. KÜSTENMACHER[1] proved the presence of cinnamic acid and cinnamyl alcohol. JAUBERT[2] identified and isolated chrysin (5,7-dihydroxyflavone) which lends its typical colour to propolis and wax. In 1911 DIETRICH[3] identified vanilline and VILLANUEVA et al.[4] identified and isolated galangine (3,5,7-trihydroxyflavone) in propolis.

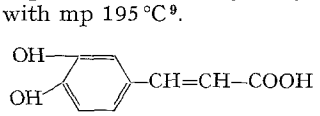
Because the number of chemical substances so far established cannot be held responsible for the rich spectrum of effects of propolis[5], our aim has been to isolate and identify further chemical substances in this bee's product.

Experiment. Isolation: 0.5 kg of finely crushed propolis was extracted at laboratory temperature, having been mixed constantly for 10 days with 1000 ml of distilled water. The extraction completed, the solution was separated from the undissolved part of propolis by filtration. A pellucid reddish-brown solution of agreeable aromatic small was obtained. Its pH was acid, between 4.5 and 5.

100 ml of this aqueous extract was extracted with 100 ml of ether. The ether was evaporated and a brownish-yellow substance was obtained having a faint aromatic smell. Its melting point was 185–190 °C. After recrystallization from hot water, the melting point rose to 195 °C and the substance decomposed at the same time. Pureness and homogeneousness of the separated crystals were controlled by thin-layer chromatography. On the base of percentages of carbon and hydrogen the summary formula was found to be $C_9H_8O_4$.

Identification. The aqueous or alcoholic solution of the isolated substance gave these characteristic chemical reactions: it reacted acidly, discoloured a solution of $KMnO_4$ and a solution of Br_2, reduced the ammonia solution of $AgNO_3$, but did not reduce Fehling's solution. With a solution of alkalies it gave an intensely yellow colour, with $FeCl_3$ a green colour which in Na_2CO_3 medium turned into a violet-blue shade: on heating, CO_2 was given off, and with dry distillation, pyrocatechine (mp 105 °C) was obtained. By means of paper chromatography (Whatman No. 1) the following Rf × 100 values were obtained: for the system ethyl-methyl keton : acetone : formic acid and water (REIO[6]), Rf 80; for the system benzene : formic acid, Rf 00; for the system 2% and 10% CH_3COOH (CHALLICE[7]), Rf 29 and 37, and for the system 20% KCl in water (HAIS[8]), Rf 30. The identification of the patch was accomplished with the help of bright blue fluorescence in UV-light at 254 and 350 nm, and with detecting agents mentioned in the individual publications.

According to these reactions and experimental factors, we deduced the presence of 3,4-dihydroxycinnamic acid I (caffeic acid) with mp 195 °C[9].



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The correctness of this conclusion was proved by paper chromatography, using as standard 3,4-dihydroxycinnamic acid mp 195 °C, the same Rf-values for both substances, and measuring the UV-spectrum of the aqueous solution of the standard and the isolated acid on the UV-apparatus Unicam (1 cm kyveta in the 185–370 cm^{-1} sphere).

Discussion. 3,4-dihydroxycinnamic acid has antibacterial activity against *S. aureus, C. diphteriae, Proteus vulgaris* × 19[10]; tuberculostatic action against *Mycobacterium tuberculosis* in vitro[11,12]; fungistatic activity against *Helminthosporium carbonum*[13], and growth inhibition in *Streptomyces scabies*[14].

It is to be expected that it is the active matter, or at least one of the active materials, in the activity of propolis against *S. aureus, Proteus vulgaris, Mycobacterium tuberculosis* and *Helminthosporium,* which has been described by LINDENFELSER[15,16] and tuberculostatic activity by FEUEREISL[17] and PRADO-FILHO[18].

3,4-dihydroxycinnamic acid is a substance which is found in many plants (KARRER[19], HÖRHAMMER[20]) from which the bees collect resin as a source of propolis. By the evidence and isolation of this acid from propolis, a further valuable argument has been gained in favour of RÖSCH's theory[21] of the origin of propolis.

Zusammenfassung. Erstmalige Isolierung von Kaffeesäure aus Propolis mittels Papierchromatographie und UV-Spektroskopie. Identifizierung als 3,4-Dihydroxyzimtsäure.

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