Phytochemistry, 1981, Vol. 20, pp. 186–187. © Pergamon Press Ltd. Printed in England. 0031-9422/81/0101-0186 $02.00/0

CAFFEIC ACID 4-β-GLUCOSIDE AS THE ACYL MOIETY OF THE *SENECIO CRUENTUS* ANTHOCYANIN

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(*Revised received* 6 *June* 1980)

Key Word Index—*Senecio cruentus*; Compositae; cineraria; acylated anthocyanin; hydroxycinammic acid–sugar derivative; caffeic acid 4-β-glucoside.

A reddish purple anthocyanin isolated from red petals of cineraria, and called rubrocinerarin (RC), has already been reported [1]. RC is cyanidin 3,7,3′-triglucoside acylated with caffeic acid, and it retains a stable purplish red colour in solution between pH 4 and 7. In this investigation, it was found that RC contains an acyl moiety other than caffeic acid. The R_f values of the new acylating moiety (**1**) were 0.58 (BAW), 0.56 (H_2O), 0.01 (*n*-BuOH-2N ammonia) and 0.57 (BEW). **1** showed purple fluorescence under UV light, changing to dull purple with ammonia vapor. **1** turned deep reddish purple with diazotized nitraniline, the colour being stable. **1** showed λmax (log ε) at 316 (sh) (4.05), 287 (4.15) and 215 (4.20) nm in 95% EtOH; 338, 286 and 254 nm in 95% EtOH–NaOH.

1 gave caffeic acid and D-glucose (1:1) on acid hydrolysis. Since **1** was unaffected by alkaline hydrolysis and was converted to methyl caffeate on heating with methanol and sulphuric acid, it must have a free carboxyl residue. 4-Hydroxy-3-methoxycinnamic acid (ferulic acid) was formed after **1** was methylated by CH_2N_2 and then hydrolysed with acid. Therefore, **1** is caffeic acid 4-glucoside.

The methyl ester of caffeic acid 4-β-glucoside was first obtained by alkaline methanolysis of the complex polymer in flax seed hulls, and is designated linocaffein [2]. The methyl ester of **1** was spectrally indistinguishable from linocaffein: 320, 291, 239 and 215 nm in 95% EtOH. In addition, **1** has been reported to be formed in the leaves of *Solanum* species and *Lycopersicum esculentum* when caffeic or *o*-coumaric acid is fed to the leaves [3]. As the acylating group of complex anthocyanins, *p*-coumaric acid is the most common and caffeic and ferulic acid are present to a lesser extent [4]. Sinapic acid [5, 6], *p*-hydroxybenzoic acid [7–9], acetic acid [10] and malonic acid [11, 12] are rarely found. To our knowledge **1** has not been previously described as an acyl group of complex anthocyanins. **1** was attached to hydroxyl group of a glucose moiety in RC since glucose acylated by **1** was produced by 0.2 N HCl hydrolysis. **1** was also found in the blue pigment isolated from cineraria (cinerarin) [13].

EXPERIMENTAL

Isolation and purification of **1**. RC was isolated from dried flowers and purified as described previously [1]. RC was treated with 8% NaOH under N_2 for 30 min at room temp. and the mixture was acidified with 4% HCl. After elimination of caffeic acid, liberated with Et_2O, the soln was concentrated *in vacuo*. The residue was dissolved in MeOH, filtered and the MeOH soln was streaked on paper (Toyo No. 514), subsequently developed with BAW. The band of **1** on the chromatogram was cut out and eluted with 80% MeOH. The eluate was concentrated and colorless needles separated after storage at 0°. After recrystallization from MeOH–H_2O, mp was 124°. (Found: C, 51.44; H, 6.00. Calculated for $C_{15}H_{18}O_{10}\cdot\frac{1}{2}H_2O$: C, 51.27; H, 5.41%) IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1650 (C=O), 1600 (C=C), 1260 and 1240 (=O–) and 1060 (C–O); ^{1}H NMR, 100 MHz (CD_3OD): δ 7.56 (1H, *d*, *J* = 16 Hz, Hα caffeic acid), δ 7.24 ~ 6.90 (3H, *m*, H-2,-5,-6 caffeic acid), δ 6.30 (1H, *d*, *J* = 16 Hz, H_β caffeic acid), δ 3.34 ~ 3.99 (4H, *m*, sugar proton); MS *m/e*: 324 (M^+), 180 (M^+ – glucose), 179, 163.

Paper- and TLC-chromatography of **1**. All R_f values were measured on Toyo No. 51 filter paper, a grade corresponding to Whatman No. 1 paper. Avicel TLC plate were also used. The solvent mixtures were: BAW (*n*-BuOH–HOAc–H_2O, 4:1:5), *n*-BuOH–2N ammonia (1:1) and BEW (*n*-BuOH–EtOH–H_2O, 4:1:2.2).

Acknowledgements—The author is grateful to Prof. Dr. H. J. Klosterman, Department of Biochemistry of North Dakota State University, for his helpful advice on linocaffein. The author wishes to thank Dr. T. Hujimori, Central Research Institute of Japan Tobacco and Salt Public Corporation, for NMR and mass spectrometric analyses, and Dr. K. Hujimori, Chemical Institute of Shinshu University, for IR spectrometric study, and his advice. Thanks are also due to Dr. T. Hayashi, Central Research Institute of Sankyo Co. Ltd. for the elemental analysis.

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Phytochemistry, 1981, Vol. 20, pp. 187–188. Pergamon Press Ltd. Printed in England.

0031–9422/81/0101–0187 $02.00/0

PHYTOALEXIN ACCUMULATION IN CHLOROFORM-TREATED COTYLEDONS OF *PHASEOLUS VULGARIS*

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(*Received* 28 *February* 1980)

Key Word Index—*Phaseolus vulgaris*; Leguminosae; isoflavonoids; phytoalexins; chloroform; elicitor.

Phytoalexins are antifungal compounds produced by plants in response to infection by micro-organisms [1, 2] and viruses [3, 4], and after treatment with various chemicals [5, 6]. Recent studies with bean, *Phaseolus vulgaris*, have suggested that when tissue is partially damaged, e.g. by infection or partial freezing [7] or by treatment with $HgCl_2$ [8], a metabolite (constitutive elicitor) is released from the damaged cells which instigates isoflavonoid phytoalexin biosynthesis in the adjacent living tissue. As part of an investigation into the relationship between death of plant cells and subsequent accumulation of isoflavonoids, we have assessed the effects of $CHCl_3$ vapour, which kills cells very rapidly, on cotyledons of *P. vulgaris*.

After exposure to $CHCl_3$ for 1–5 min, cotyledons showed a gradual browning of their surfaces after incubation for between 10 and 72 hr. Isoflavonoids accumulated in the discoloured cotyledons and the amounts of phytoalexin produced became greater as the damage progressed (Table 1). Phaseollin, phaseollinisoflavan and kievitone were most abundant, being present at concentrations up to 96 μg/g cotyledon. Similar quantities of phaseollin were produced by cotyledons treated with $HgCl_2$ [8]. Phaseollidin and 2 hydroxygenistein were also detected (UV and MS [9]); however, their concentrations were always less than 3 μg/g cotyledon. Cotyledons treated with $CHCl_3$ for more than 10 min became totally flaccid and did not become pigmented or produce any of the above compounds. No isoflavonoids were detected in undamaged cotyledons.

Chloroform is very volatile and is unlikely to persist within the treated tissues. The results therefore support the suggestion, made earlier, following studies with $HgCl_2$ [8], that the accumulation of phytoalexins can be a direct consequence of the death of superficial cells of bean cotyledons.

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