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A NEW TYPE OF GLYCOFLAVONOID FROM VITEX LUCENS

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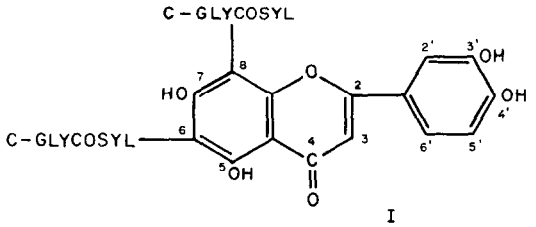
We propose formula I for the pigment lucenin-1, which is the first example of a new structural type of glycoflavonoid.



I

Lucenin-1 was isolated and crystallized from extractives of the New Zealand wood, Vitex lucens. Previous work on this species had indicated that in addition to the classical glycoflavonoids, vitexin and saponaretin (1,2), and a vitexin-O-glycoside (3), a new series of flavonoid

* Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

carbon-glycosides was present (3,4). This paper describes the isolation of one member of this new series and presents the following evidence to support the hypothesis (4) that the substance is a 6,8-di-C-glycosylluteolin, I.

Initial paper chromatographic studies* disclosed that lucenin-1 had R_f (x 100) values of 34 in butanol-27% acetic acid (1:1) (solvent A) and 16 in 3% aqueous sodium chloride (solvent B). These mobilities were lower than those observed for previously described glycoflavonoids and their O-glycosides in solvent A and for the O-glycosides in solvent B. For example, in solvent A orientin (luteolin-8-C-glucoside (5)) and vitexin-x-O-xyloside have R_f values of 45 and 63 on the same sheet; in solvent B, they had values of 5 and 46. These comparative data suggested that lucenin-1 had more hydroxylation and/or higher molecular weight.

Ultraviolet spectral studies, including diagnostic shifts (6), gave curves superimposable on those of the luteolin glycoflavonoids previously studied (7) and of luteolin. Thus, the flavonoid skeleton appeared to be that of luteolin (3',4',5,7-tetrahydroxyflavone), and the shifts demonstrated that the four hydroxyl groups were present in the OH form.

Subjecting the material to typical acidic hydrolytic conditions produced no sugar, so lucenin-1 is not an O-glycoside. On the other hand, a proliferation of similar flavonoid spots on the chromatograms was observed; this result is reminiscent of, but more extensive than, those obtained with vitexin and other glycoflavonoids similarly treated. From Sephadex columns, lucenin-1 and related compounds present in crude extracts elute simultaneously with known glycoflavonoid O-glycosides and before their aglycons, a fact suggesting similarity in molecular weights.

Lucenin-1 was isolated in crystalline form from cold, methanolic extracts of heartwood of V. lucens by the following steps: (a) Precipitation with neutral lead acetate; (b) separation from other glycoflavonoids

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* Work of Juliana H. S. Chow in 1960, Wellesley College.

including lucenin-3 on a ChroMax-pressurized, rolled paper column with solvent A; (c) freeze drying of the eluate; (d) separation from lucenin-2 and lucenin-4 on a second column developed with 5% acetic acid; (e) freeze drying; (f) slow crystallization of chromatographically pure material by treatment with minimum amounts of water; (g) removal of iron contaminants by repeated extraction into cold ethanol; and (h) recrystallization from water. The resultant pale yellow, transparent crystals lost solvent in the air and decomposed at about 230°.

Anal. Calcd. for $C_{27}H_{30}O_{16}$ (di-C-glucopyranosylluteolin); %C, 53.13; %H, 4.95; and M.W. 610. Found: %C, 53.03; %H, 5.44; and M.W. 573 ±10%.

The analytical data is in accord with a di-C-glycosyl derivative in which the glycosyl groups are in the form most recently proposed for the C-glycosyl derivative of the co-occurring vitexin (8).

The n.m.r analysis of lucenin-1 was determined by first converting it into the trimethylsilyl ether by the previously described procedure (9,10). In the resultant spectrum* of this derivative, four proton signals could be assigned to the flavonoid nucleus. Of these, three displayed typical signal patterns for B-ring protons (9,10): H-2', doublet (J = 2 c.p.s.) at τ 2.78; H-5', doublet (J = 8 c.p.s.) at τ 3.10; and H-6', poorly resolved quartet (J_{meta} = 2 c.p.s., J_{ortho} = 8 c.p.s.) at τ 2.27 c.p.s.

A singlet near τ 3.72, integrating for one proton, occurred in the region expected for either a C-6 or C-8 A-ring proton or a C-3 proton. This signal could be unambiguously assigned to the C-3 proton by the following reasoning: It was previously noted (9,10) that (a) the hydroxyl group at C-5 trimethylsilylated only slowly when C-6 substituents were present; (b) in the trimethylsilyl ether of a flavone having a free C-5 hydroxyl group,

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* All spectra were measured in carbon tetrachloride on a Varian A-60 spectrometer with tetramethylsilane as an internal reference.

the C-3 proton signal occurred about 0.15 p.p.m. downfield from its position in the spectrum of the totally trimethylsilylated flavone (this shift is characteristic for the C-3 proton in flavones); and (c) the C-5 hydroxyl proton, hydrogen bonded to the carbonyl, could be detected by its signal near τ -3. The n.m.r. spectrum of partially trimethylsilylated lucenin-1, obtained by a 10-minute trimethylsilylation reaction time, displayed a signal at τ -3.5 and a singlet at τ 3.57, each integrating for about one proton. In the spectrum of the totally trimethylsilylated lucenin-1, obtained by a 4-hour reaction time, the τ -3.5 signal was not present, and the singlet had shifted to τ 3.72, thus confirming the assignment of this signal to the C-3 proton. The n.m.r. spectra are in accord with a 6,8-disubstituted luteolin, since there are no protons on the A-ring.

The n.m.r. signals for the sugar moiety in I, integrating roughly for 14 protons between τ 5 and 6.9, combined in many respects the signals observed in this same region for the n.m.r. spectra of the trimethylsilyl derivatives of vitexin (apigenin-8-C-glucoside) and saponaretin (apigenin-6-C-glucoside); this suggests a luteolin-6,8-di-C-glucoside as one likely structure possibility for lucenin-1.

As mentioned above, lucenin-1 occurs in V. lucens as a member of a series of glycoflavonoids. Running as contiguous spots on two-dimensional chromatograms are lucenin-2 and lucenin-3 (originally called the A-B group (3,4)), lucenin-4 and lucenin-5. These have ultraviolet spectra superimposable with lucenin-1. It has now been shown that, in hot acid, at least lucenins-1, -3, and -4 form an equilibrium mixture, and it is postulated that they are all 6,8-diglycosyl derivatives, differing only in the structure of the glycosyl groups. A second group of chromatographically observable spots (originally called the C spots (3,4)), appear to be derivatives of apigenin analogous to the lucenin series and have been designated as vicenins-1, -2, and -3.

A paper describing in detail the isolation and characterization of 13 glycoflavonoids of V. lucens is in preparation.

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