Identification of 3′,4′,7-Trihydroxyflavone in Ladino Clover

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A yellow crystalline flavone, $C_{15}H_{10}O_5$, previously isolated from ladino clover (*Trifolium repens*), has now been identified as 3′,4′,7-trihydroxyflavone. This structure was confirmed by comparing the ultraviolet and infrared spectra and melting point of the unknown to those of an authentic synthetic sample.

PHENOLIC COMPOUNDS of forages are under investigation at this laboratory. To date, the estrogenic compound, coumestrol (1); the related coumestan, trifoliol (2); the estrogenic isoflavones, genistein, daidzein, biochanin A, and formononetin (3); the dicoumaryl ether, daphnoretin (4); and the flavone, tricin (5), have been isolated and identified.

Recently, the isolation of 17 phenolic compounds from ladino was reported (6). One of these, compound XVII, having the empirical formula $C_{15}H_{10}O_5$, has now been identified. The formation of a triacetate indicated the presence of three phenolic hydroxyl groups. The similar ultraviolet spectra of compound XVII triacetate ($\lambda_{max.}$ 299, 253) and of the unsubstituted compound, flavone itself ($\lambda_{max.}$ 297, 250), suggested that the unknown was also a flavone (7). Production of orange-red color, upon treatment of an alcoholic solution of the parent compound with magnesium and hydrochloric acid, further confirmed its flavone structure (8).

A marked shift in the ultraviolet spectrum of the parent compound in the presence of boric acid–sodium acetate solution indicated an *o*-dihydroxyl substitution (9). A comparison of the melting points of the phenolic compound and its triacetate to those reported in the literature for known trihydroxyflavones indicated that it might be 3′,4′,7-trihydroxyflavone.

This structure was unequivocally confirmed by comparing the spectra and mixed melting points to those of authentic samples. Although the synthesis of this compound has been described (10), this is the first report (to our knowledge) of its occurrence in a natural plant product.

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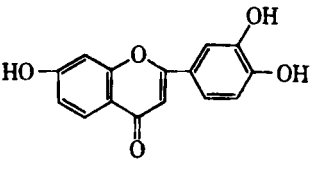

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EXPERIMENTAL

A paper giving the details of the isolation of this yellow crystalline compound (compound XVII) by countercurrent distribution from an acetone extract of ladino clover was recently prepared (6). The flavone was recrystallized from methanol to yield the analytical sample, m.p. 318–322° dec.; no depression in melting point with a synthetic sample, m.p. 318–322° dec. $\lambda_{max.}^{EtOH}$ 342 (log ϵ, 4.33), 313 (sh) (log ϵ, 4.18), 236.5 (log ϵ 4.31) mμ. (Spectrum identical to that of synthetic 3′,4′,7-trihydroxyflavone.) The infrared spectra of the isolated and synthetic flavones were also identical.

Anal.—Calcd. for $C_{15}H_{10}O_5$: C, 66.7; H, 3.70. Found: C, 66.8; H, 3.65.

Thirty milligrams of the parent phenol was treated in the usual way with acetic anhydride and fused sodium acetate to form the triacetate. The triacetate was recrystallized from acetone to give 28 mg. of white needles, m.p. 209–210°; no depression in melting point with a synthetic sample, m.p. 209–210°. $\lambda_{max.}^{EtOH}$ 299 (log ϵ, 4.43), 253 (log ϵ, 4.25) mμ. The ultraviolet and the infrared spectra of the natural and synthetic acetates were identical.

Anal.—Calcd. for $C_{21}H_{16}O_8$: C, 63.8; H, 4.04; CH_3CO, 32.6. Found: C, 63.6; H, 4.414; CH_3CO, 32.8.

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