3′,4′,7-Trihydroxyflavone in Alfalfa

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A yellow crystalline flavone previously isolated from alfalfa has now been identified as 3′,4′,7-trihydroxyflavone. This structure was confirmed by comparison of the ultraviolet and infrared spectra and melting point of the unknown with those of an authentic synthetic sample.

A RECENT paper described countercurrent distribution (CCD) procedures for the isolation of coumestrol and 12 other phenolic compounds from alfalfa (1). About a milligram of a compound, designated there as compound V, was obtained by recrystallization of one of the fractions obtained by countercurrent distribution of the crude crystalline coumestrol preparation.

The appearance of color on paper chromatograms under ultraviolet light, with and without ammonia vapor (1), as well as mixed, two-dimensional paper chromatograms in our standard solvent systems suggested that compound V might be identical with 3′,4′,7-trihydroxyflavone previously isolated from ladino clover (2). To verify this, more of this compound was isolated by column chromatography. It has now been unequivocally confirmed to be 3′,4′,7-trihydroxyflavone by comparing its spectra and mixed melting points with those of an authentic sample.

EXPERIMENTAL

Details of the isolation of this yellow crystalline compound (compound V) from alfalfa by countercurrent distribution followed by recrystallization of one of the resulting fractions from the CCD separations (fraction 15) were presented earlier (1). However, insufficient material was obtained at that time for elemental analysis or physical measurements.

To obtain a larger supply of compound V, fraction 15 from CCD through solvent system B (*Reference 1*, Fig. 2), which consisted of about 14 Gm. of a tarry mixture, was taken up in a minimum of acetone and added to silica gel (Grace Davison). This mixture was dried and added to the top of a silica gel column (7 × 17 cm.). The column was eluted with increasing amounts of methanol in chloroform. The fraction that was eluted with 2% methanol in chloroform contained mostly compound V plus a small amount of several other fluorescing compounds. This fraction was rechromatographed in the same manner on silica gel. The column was again eluted with increasing amounts of methanol in chloroform and 1-L. fractions were taken. Fractions 9–12, eluted with 3% methanol in chloroform, were taken to dryness and recrystallized from methanol and chloroform. Yellow needle crystals (280 mg.) were collected. An analytical sample was prepared by twice recrystallizing from methanol, m.p. 331–332°, undepressed upon admixture with an authentic sample. Ultraviolet and infrared spectra were also identical.

Anal.—Calcd. for $C_{15}H_{10}O_5$: C, 66.7; H, 3.70. Found: C, 66.5; H, 3.81.

Compound V Acetate.—Compound V (50 mg.) was acetylated with acetic anhydride and sodium acetate in the usual manner. Recrystallization of the acetate from acetone gave crystals (55 mg.), m.p. 214.5°, undepressed upon admixture with an authentic sample. Ultraviolet and infrared spectra were also identical.

Anal.—Calcd. for $C_{21}H_{16}O_8$: C, 63.8; H, 4.04; CH_3CO, 32.6. Found: C, 63.7; H, 4.13; CH_3CO, 32.9.

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Communications

Determination of the Physical Safety Factor of Potential Pharmacological Agents

Sir:

In the pharmacological evaluation of drugs, the intravenous route of administration is frequently employed. A chance observation of precipitation of the drug TA-PA260 (an antibiotically active triacetyloleandomycin derivative), when the solution was administered intravenously to an anesthetized dog, resulted in the consideration that many of the observed and measured effects of drugs are erroneously interpreted as being due to the pharmacological effects of the drug rather than to the possible change in the physical state of the drug.

To avoid this possibility of false data, a procedure has been developed in which the solubility of drug solutions in blood plasma is measured. The maximum solubility of the drug in blood