3′,4′,7-Trihydroxyflavone in Alfalfa

By E. M. BICKOFF, S. C. WITT, and A. L. LIVINGSTON

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

plasma, called the maximum safe concentration (MSC), is obtained by adding fixed volumes of varying concentrations of the drug solution to fixed volumes of blood plasma. Observations are then made of the treated plasma for the level of precipitation or flocculation. The MSC is thus experimentally determined as the maximum drug concentration not exhibiting precipitation or flocculation. Greater sensitivity could be achieved using spectrophotometric techniques. This measurement can be utilized in the suggested equations for the determination of the solubility of the solution when the drug is to be given by the intravenous route.

Utilizing several assumptions, it is possible to calculate the maximum safe intravenous dose (MSD) of a test compound. The MSC and MSD are in reference to the physical rather than the pharmacological safety factors of the compound. That is, these factors indicate the maximum concentration and dose of the drug solution which can be administered intravenously with some assurance that the drug will not precipitate in the circulating blood.

The following assumptions are necessary for the calculations of the MSD in dogs. (*a*) The total circulating blood volume is 6 to 8 (average 7)% (1) of the animal body weight. (*b*) During the period of infusion, the drug is contained within the circulating blood volume. (*c*) Since the minimum time necessary for the equidistribution of a compound throughout the circulating blood volume is 3 min. (2), based upon distribution of Evans Blue Dye (T-1824), it is necessary that the time of continuous constant rate of intravenous infusion be at least 3 min. (*d*) The equilibration of a drug in blood plasma is essentially equivalent to that for Evans Blue Dye.

To correct for variations in intravenous infusion time, a constant is obtained which utilizes the MSC and the infusion time as follows:

$$K_c = \text{MSC}\,(X/A)$$

where MSC is the maximum safe concentration, A is the equidistribution circulation time of 3 min., and X is the actual time of infusion. When $X > 3$, then $X/A = 1$. The K_c value, in mg./ml. solution, can then be used to calculate the maximum safe intravenous dose.

Since, as was assumed, the circulating blood volume is 7% of the body weight, then the maximum safe intravenous dose would be calculated as:

$$\text{MSD} = K_c \cdot 70$$

in mg./Kg. body weight.

This concept was examined with the drug TA-PA260. This compound in the free base form is insoluble in water but readily dissolves when neutralized with aqueous hydrochloric acid solution to a pH of 5 where its salt form remains in solution. In anesthetized dogs, a 20-min. intravenous infusion of 100 mg./Kg. of TA-PA260 produced an increased mean arterial pressure of 74 mm. Hg and was associated with marked retching episodes during the infusion period. Thirty minutes later a second infusion of 100 mg./Kg. of this drug did not produce the blood pressure change and the retching previously observed. Examination of the femoral vein into which the infusion was made revealed a large amount of precipitant which conceivably could have been responsible for the differences in responses.

Plasma solubility studies on TA-PA260 revealed that this compound had a MSC of 1.25 mg./ml. Since the infusion time was greater than 3 min., correction for equidistribution time was not necessary, and thus K_c also was 1.25 mg./ml. of blood. The MSD, calculated to be 87.5 mg./Kg., indicates that the intravenous administration of doses of TA-PA260 as high as 87.5 mg./Kg., with infusion time greater than 3 min., should not be expected to change in physical state, and therefore, the responses observed are most likely the pharmacological effects of the drug. When, however, an intravenous dose of TA-PA260 exceeding 87.5 mg./Kg. is administered, then one must be cognizant of the possibility that some of the observed effects might be due to a change in the physical state of the drug.

These considerations have possible implications in every phase of intravenous pharmacological evaluation of drugs which are insoluble at plasma pH. Even compounds soluble in solutions of physiological pH may precipitate out of the blood plasma, as for example by protein binding and, therefore, should be included in any subsequent solubility measurements.

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B. Korol
W. J. Lang

Department of Psychiatry
Missouri Institute of Psychiatry
University of Missouri
St. Louis

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