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SHORT COMMUNICATION

IDENTIFICATION OF 7,4'-DIHYDROXYFLAVONE IN FORAGES

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Abstract—7,4'-Dihydroxyflavone, a previously unrecognized constituent of plants, has been found in alfalfa (*Medicago sativa*) and in ladino clover (*Trifolium repens*).

INTRODUCTION

The occurrence of only two dihydroxyflavones in nature has been reported so far: chrysin (5,7-dihydroxyflavone), first reported in 1873 in the buds of poplar,[1] and primetin, the 5,8-homologue.[2] 7,4'-Dihydroxyflavone had never been found in nature, although it was first synthesized in 1899.[3] Pratol,[4] isolated in 1910 from *Trifolium pratense*, was originally thought to be the 4'-methyl ether of this compound. However, recent studies[5,6] make it almost certain that pratol must be 7-hydroxy-4'-methoxy isoflavone (formononetin) instead. The present communication describes the identification of 7,4'-dihydroxyflavone in both alfalfa (*Medicago sativa*) and ladino clover (*Trifolium repens*).

EXPERIMENTAL AND RESULTS

Recently the isolation of 13 phenolic compounds from alfalfa was reported.[7] One of these (compound IV) had the empirical formula $C_{15}H_{10}O_4$ (Found: C, 70·8; H, 3·99. Calc. for $C_{15}H_{10}O_4$: C, 70·9; H, 3·93% and m.p. 315–316°. A compound having the same empirical formula (Found: C, 70·6; H, 4·08%) and m.p. was found in ladino clover.[8] This compound (No. VI) was isolated from an acetone extract of ladino clover by countercurrent distribution. Infrared spectra and mixed melting point determination showed that the two compounds were identical. Formation of a diacetate by refluxing with acetic anhydride and fused sodium acetate indicated the presence of two hydroxyl groups (Found: C, 67·5; H, 4·17; acetyl, 24·9. Calc. for $C_{19}H_{14}O_6$: C, 67·5; H, 4·14; acetyl, 25·5%.)

On paper chromatograms under u.v. light the parent compound showed an intense yellow color, which changed to a bright green-yellow with ammonia vapor. Production of an orange-red color when an alcoholic solution of the compound was treated with magnesium

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and hydrochloric acid (the cyanidin test) indicated that it might be a flavone.[9] This was substantiated by the u.v. spectra of the compound and its acetate. The u.v. spectrum of the latter was very similar to that of flavone itself,[10] and the parent phenol showed absorption peaks at 225 and 329 mμ and a shoulder at 253 mμ. A pronounced bathochromic shift in the u.v. spectrum of band II of the parent compound from 225 to 267 mμ upon the addition of sodium acetate[11] indicated the presence of a hydroxyl group in the 7 position. Alkaline fusion of the compound following a previously described procedure[12] yielded resorcinol, beta-resorcylic acid, resacetophenone and *p*-hydroxy benzoic acid, suggesting that the compound was 7,4′-dihydroxyflavone. Final confirmation of the structure was obtained by comparison of the u.v. and i.r. spectra and by determination of mixed m.p. with an authentic sample of 7,4′-dihydroxyflavone.

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