and then concd under red. press. The residue was re-extrd in EtOH, and examined by PC in *n*-BuOH–HOAc–H_2O (4:1:5). The biflavonyls appeared under UV as a dark absorbing band immediately behind the solvent front, and fluoresced dark yellow on addition of $AlCl_3$ indicating the presence of flavonoid type compounds. The band was eluted with 1% HOAc in 70% EtOH concd and re-chromatographed on pre-coated Si gel plates developed in C_6H_6–pyridine–HCO_2H (BPF) (100:20:7), revealing the mixture of biflavonoid bands present in the extract as a number of dark, UV-absorbing bands. Each band was extrd individually and a final sepn of each was carried out on pre-coated cellulose plates developed in fresh prepared *n*-BuOH–2 N NH_4OH, 1:1 (upper layer) (BN). Initial identification was made by co-chromatography with authentic samples of amentoflavone, 4‴ monomethyl amentoflavone and 7″4‴ dimethyl amentoflavone in BPF and BN. Confirmation of the identification of these compounds as amentoflavone-based ethers was provided by partial methylation and permethylation (CH_2N_2) of some individual bands and some eluants. The methylated eluants provided only the characteristic hexamethyl ether of amentoflavone, which co-chromatographed with an authentic sample in BPF.

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SYNTHESIS AND STRUCTURAL PROOF OF WAIROL, A NEW COUMESTAN FROM *MEDICAGO SATIVA*

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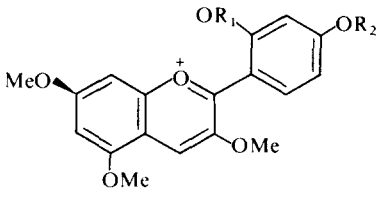
Key Word Index—*Medicago sativa*; Leguminosae; synthesis; 3-hydroxy-7,9-dimethoxycoumestan; wairol.

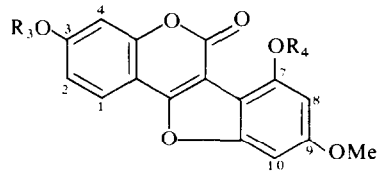
Abstract—Wairol, a coumestan from *Medicago sativa*, has been synthesized and its structure thereby has been confirmed.

In a previous investigation [1], a new coumestan was isolated from fungal-infected lucerne foliage, and tentatively identified as 3-hydroxy-7,9-dimethoxycoumestan (wairol, **3**). In this paper we report the chemical synthesis of wairol, confirming the structure previously assigned, and report some physical characteristics of this compound.

The suitably protected flavylium salt, 3,5,7-trimethoxy-2′,4′-dibenzyloxyflavylium chloride (**1**) was prepared by acid-catalysed condensation of 4,6-dimethoxysalicylaldehyde with 2,4-dibenzyloxy-ω-methoxyacetophenone [2] (76%), red needles, λ_{max} (EtOH–0.5% HCl) nm (log ε) 512 (3.91), 276 (4.12) (Found: C, 70.46; H, 5.37; $C_{32}H_{29}O_6Cl$ requires: C, 70.58; H, 5.33%.)



1 $R_1 = R_2 = Bz$
2 $R_1 = R_2 = H$



3 $R_3 = H$, $R_4 = Me$
4 $R_3 = R_4 = H$
5 $R_3 = R_4 = CD_3$
6 $R_3 = CD_3$, $R_4 = Me$
7 $R_3 = Ac$, $R_4 = Me$

Acid hydrolysis of **1** with HCl in HOAc gave 3,5,7-trimethoxy-2′,4′-dihydroxyflavylium chloride (**2**) (54%), red-brown precipitate, λ_{max} (EtOH–0.5% HCl) nm (log ε) 516 (4.28), 290 (4.05), 276 (4.19) (Found: C, 47.53; H, 3.71; $C_{18}H_{17}O_6Cl$ requires: C, 47.57; H, 3.74%.) Wairol was synthesized by H_2O_2 oxidation of **2** followed by acid-catalysed lactonization [2], plates (57%) mp 292–294° (Me_2CO–MeOH), λ_{max} (EtOH) nm (log ε) 347 (4.26), 302 (3.78), 266 (4.25), 216 (4.34). ν_{max} (nujol) cm^{-1} 3180, 1705, 1620, 1310, 1290, 1260, 1225, 1140, 1085, 1010, 953, 808, 770, 725. The product was indistinguishable from the natural compound by TLC and MS.

Treatment of **3** with Ac_2O–pyridine gave 3-acetoxy-7,9-dimethoxycoumestan (**7**), needles, mp 238–242° (EtOH), MS (rel. int.) m/z 354 (M^+, 33), 312 (100), ^1H NMR (60 MHz, DMF-D_7, 115°) δ 8.03 (1 H, *d*, J = 8 Hz, C-1), 7.36 (1 H, *s*, C-4), 7.27 (1 H, *d*, J = 8 Hz, C-2), 7.02 (1 H, *d*, J = 1.5 Hz, C-10), 6.67 (1 H, *d*, J = 1.5 Hz, C-8). The ^1H NMR of **7** compares well with that of trifoliol (**4**) [3].

We previously suggested [1] that the prominent fragment ion at m/z 283 ($M^+ - CHO$) in the MS of **3** was diagnostic for an *O*-methyl substituent on C-7. In order to determine the regiospecificity of this fragmentation, the mass spectra of **5** and **6** prepared from **4** and **3** respectively with CD_3I were examined. Results indicated that only the MS of **5** showed an intense ($M^+ - CDO$) fragment ion, confirming the earlier conclusion that this fragmentation pathway probably involves the lactone carbonyl and the C-7 *O*-methyl group.

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PREPARATION OF CHACONINES BY ENZYMIC HYDROLYSIS OF POTATO BERRY ALKALOIDS

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; enzymes; glycoalkaloids; steroids; chaconine.

Abstract—Endogenous enzyme activity in a blend of potato berries and blossoms converts the contained glycoalkaloids within 24 hr to a mixture of α-solanine and β_2-chaconine, the latter a product of the conversion of α-chaconine by cleavage of the rhamnose on C_2^1 of the dirhamnoglucoside. The β_2-chaconine was isolated by ethyl acetate fractionation of the crude glycoalkaloid precipitate. α-Chaconine can be obtained after heat-destruction of enzyme activity in the same plant tissues.

INTRODUCTION

The triglycosidic glycoalkaloids α-solanine and α-chaconine are present in all tissues of the cultivated potato, *Solanum tuberosum* [1, 2]. From these, the di- and monoglycosides can be obtained by partial acid hydrolysis and column chromatography (CC) on alumina [1, 3]. α-Solanine is readily isolated from potato sprouts by repeated crystallization of crude glycoalkaloid from ethanol [4] but there is little information on isolation of chaconines outside the classical work of Kuhn and Löw [3] with *Solanum chacoense*. The increasing knowledge on glycoalkaloid hydrolases opened a path for investigating the potential use of endogenous enzymes in tissue homogenates for the preparation of chaconines.

The pattern of glycoalkaloid hydrolysis by enzymes has been already reported for sprouts, foliage, blossoms and dormant tubers [5–7] but not for potato berries. The existence of a rhamnosidase which attacks α-chaconine in blossoms, reaffirmed recently [7], was also shown to apply to berries [8] in which the hydrolysis proceeds more rapidly than in blossoms since berries are found infrequently on most potato cultivars and are low in glycoalkaloids (0.07–0.10% fr. wt) as distinct from blossoms (0.6–0.9% fr. wt) a blend of the two tissues was investigated as a potential means of obtaining chaconine metabolites.

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