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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RESULTS AND DISCUSSION

A blend of potato berry and blossom homogenates, which contain approximately equal amounts of α-solanine and α-chaconine, showed a rapid and complete conversion of the latter to β_2-chaconine at pH 6 within 24 hr at 37°. Without buffer (deionized water homogenate) the hydrolysis was incomplete in this time. β_2-Chaconine was the only new metabolite in the digest and α-solanine remained intact. Only after prolonged incubation (96 hr) were traces of solanidine found. The 24-hr incubation resulted in a simple two-component glycoalkaloid mixture of α-solanine and β_2-chaconine. The two substances can be chromatographically separated (TLC R_f 0.26 and 0.55, respectively) but it is simpler to use their variation in solubility in ethyl acetate. Heating the crude glycoalkaloid mixture in ethyl acetate brings only β_2-chaconine into solution (*ca* 220 mg/100 ml). Similarly, addition of acetate to an alcoholic solution of the glycoalkaloid will precipitate α-solanine quantitatively from the mixture provided that no α-chaconine is present.

The hydrolysis of α-chaconine to β_2-chaconine was attributed to the action of a specific rhamnosidase capable of removing rhamnose from C_2^1 of the glucoside. Hence, the name of chaco-2-α-rhamnosidase is proposed for this enzyme, the chacotriose glucoside (2^1,4^1-dirhamnoglucoside) being the substrate. β_1-Chaconine observed in hydrolysates of dormant potato tubers [7] must be a product of another enzyme system active at the C_4^1 site. The presence of chaco-2-α-rhamnosidase in blossoms and berries renders these tissues eminently suitable for β_2-chaconine preparation. The monoglucoside, γ-chaconine, can then be easily obtained *via* mild acid hydrolysis which yields a mixture of α-chaconine and solanidine. Potato sprouts are unsuitable for β_2-chaconine preparation due to the production of β-solanine in the homogenates.

α-Chaconine can be isolated from a mixture with α-solanine which is obtained from blossoms by first destroying the enzyme by heat. The mixture is fractionated from EtAc–EtOH–5% aq. NH_4OH (80:16:4) in which the α-solanine is less soluble.

EXPERIMENTAL

Plant material. Berries of potato seedling B5141-6 and blossoms of cv Kennebec were collected and kept at −20° until required.

Homogenate incubation. Homogenates were prepared at 1:4 dilution (w/v) in McIlvaine buffer, pH 6.0.

Glycoalkaloid determination. Test samples were extracted with 20 ml 1% HOAc, precipitated twice with conc. NH_4OH, centrifuged, and the ppt. dissolved in 2 ml 0.5% HOAc for TLC according to ref. [9], detection by 25% $SbCl_3$ in glac. HOAc (w/w). Concentrations of glycoalkaloid were estimated visually by reference to a calibrated plate with 0.02–2.0 μg α-chaconine. Each TLC plate was run with two reference samples, 2 μg of α-S. α-Ch. β_2-Ch, and SDine. A clear plate over the calibrated plate, silicone-sealed and kept in the dark is useable up to 10 days. Glycoalkaloid recovery was also estimated by colorimetry with 80% H_2SO_4 + 1% HCOH reaction at 575 nm.

Identification. Pure α-S and α-Ch were separately acid-hydrolysed [1, 3] and resolved by TLC; a 45-min mix of hydrolysates, useable as reference contains all derivatives separable on a single TLC run: α-S, α-Ch, β_2-S, β_1-Ch, β_2-Ch, γ-S, γ-Ch, and SDine; α-Ch was isolated on dry Al_2O_3 column [2, 3]; α and β_2-chaconine were identified also by IR spectra (KBr), mps, and $[\alpha]_D^{20}$ (pyridine; $c = 1.00$), and by TLC of sugars in acid hydrolysates.

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