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FLAVONOID GLYCOSIDES OF *LOTUS CORNICULATUS* (LEGUMINOSAE)

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Key Word Index—*Lotus corniculatus*; Leguminosae; flavonoids; flavonol glycosides.

Abstract—Two-dimensional TLC of stems and leaves of *Lotus corniculatus* revealed the presence of *ca* 25 flavonoid glycosides. Among these, 14 were identified; 10 are new for this species. This pattern is qualitatively the same among different populations of this plant but the relative amounts of mono- and diglycosides varies considerably from one population to another.

INTRODUCTION

Lotus corniculatus L., which has a widespread distribution throughout the world, is used for forage production [1, 2]. Previous studies on the leaf flavonoid glycosides of this plant [3, 4] indicated the presence of five compounds quercetin 3-rhamnoside, quercetin 3,7-rhamnoglucoside, quercetin 3-rhamnoglucoside, kaempferol 3,7-dirhamnoside and kaempferol 3,7-rhamnoglucoside. In the present study, 2D-TLC has been applied to the aerial parts of *L. corniculatus* (stems and leaves only): 25 spots due to flavonoid glycosides were revealed; 14 of these were isolated and identified [5, 8].

RESULTS AND DISCUSSION

As shown in Fig. 1, we have isolated and identified: kaempferol 7-rhamnoside (m1), kaempferol 3-rhamnoside (m2), kaempferol 3-glucoside (m3) sexangularetin 3-glucoside (m4), quercetin 7-rhamnoside (m5), quercetin 3-rhamnoside (m6), quercetin 3-galactoside (m7), corniculatusin 3-galactoside (m8), corniculatusin 3-glucoside (m9), kaempferol 3,7-dirhamnoside (d1), kaempferol 3-rhamnoside - 7 - glucoside (d2), sexangularetin 3 - rhamnoside - 7 - glucoside (d3), quercetin 3,7-dirhamnoside (d4) and quercetin 3 - rhamnoside - 7 - glucoside (d5). Ten products are mentioned for the first time in this species. Their identification was realized through their chromatographic and spectrophotometric properties before and after acid hydrolysis. The sugars were identified by GC of their silylated derivatives; the number and position of sugars were determined by UV spectroscopy, and by chromatographic comparison with literature and reference samples [9].

As in previous investigations, only flavonol glycosides were found but there are significant variations in glycosidic patterns.

A study of the glycosidic patterns of different populations indicated that all *L. corniculatus* populations have the same qualitative flavonoid glycosidic pattern (for the 14 compounds identified). However, there are important quantitative variations in the relative content of di- and monoglycosides from one population to another; some have a greater digly-

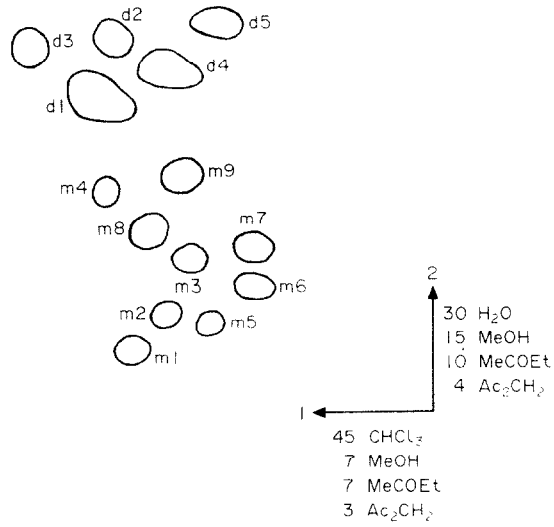

Fig. 1. Two-dimensional TLC on Polyamide of *Lotus corniculatus* leaf flavonoids. m, Monoside; d, dioside; m1, kaempferol 7-rhamnoside; m2, kaempferol 3-rhamnoside; m3, kaempferol 3-glucoside; m4, sexangularetin 3-glucoside; m5, quercetin 7-rhamnoside; m6, quercetin 3-rhamnoside; m7, quercetin 3-galactoside; m8, corniculatusin 3-galactoside; m9, corniculatusin 3-glucoside; d1, kaempferol 3,7-dirhamnoside; d2, kaempferol 3-rhamnoside-7-glucoside; d3, sexangularetin 3-rhamnoside-7-glucoside; d4, quercetin 3,7-dirhamnoside; d5, quercetin 3-rhamnoside-7-glucoside.

cosidic pool, while others a greater monoglycosidic pool. For example, the ratio diosides–monosides reaches 14.8 for a population from la Sauvetat (1200 m, in the Massif Central) and only 0.5 for a population from the Col d'Izoard (2630 m, in the Alps). Our further aim is to correlate this glycosidic variation with differences in ploidy level with contrasting habitats and with phytogeographical distribution.

It must be noted that the presence of 8-methoxyflavonols (corniculatusin and sexangularetin) has never been reported before in the stems and leaves of *L. corniculatus* but they are well known to occur in the flowers [4, 10, 11]. Their identification in this work may be explained by the presence of yound flower buds in the leaf material studied.

EXPERIMENTAL

Plants were collected in France and voucher specimens are deposited in our laboratory. Air-dried, ground aerial parts (leaves and stems, 1000 g) were extracted × 3, at room temp., with 70% aq. MeOH. The MeOH–H_2O extracts were evaporated and the residue was dissolved with boiling H_2O. When cold, the H_2O-soluble fraction was filtered, then extracted several times with EtOAc. The EtOAc extracts were evaporated to dryness and dissolved in MeOH. This MeOH extract was fractionated using a combination of CC (Sephadex LH-20 with MeOH, then Polyamide SC-6 with H_2O gradually enriched with MeOH) and 1D-PC (Whatman No. 3) in HOAc–H_2O (3:17). Compounds, when not pure, were separated again by prep. TLC on Polyamide DC-11 (H_2O–MeOH–MeCOEt–Ac_2CH_2, 15:2:2:1 or $CHCl_3$–MeOH–MeCOEt–Ac_2CH_2, 6:3:2:1). Chromatographic bands were eluted with MeOH. The identity of flavonoid glycosides was determined by chromatography together with authentic samples and by UV spectrophotometry, before and after acid hydrolysis. Hydrolysis was performed with 2 N HCl at 100° for 10–90 min according to the position of sugars. For the compounds d_2, d_3 and d_5, the location of the sugars was determined after a partial hydrolysis by 0.5 N HCl for 2 or 3 min. Sugars were identified by GC after silylation with C_5H_5N and B.S.T.F.A. + 1% HMDS on Gas Chrom Q, 80–100 mesh, with 5% SE 52. 2D-TLC on polyamide DC-11 was performed with the solvents: $CHCl_3$–MeOH–MeCOEt–Ac_2CH_2 (15:2:2:1) and H_2O–MeOH–MeCOEt–Ac_2CH_2 (6:3:2:1). The position of the flavonoid glycosides on the 2D-TLC was ascertained by elution of the different spots and co-chromatography with authentic samples.

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