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MALONATED FLAVONOL 3-GLUCOSIDES IN *CICER ARIETINUM*

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Key Word Index—*Cicer arietinum*, Leguminosae, chickpea, flavonol 3-(malonylglucoside), kaempferol 3-(malonylglucoside), kaempferol 3-(apiosyl-malonylglucoside)

Abstract—Re-examination of the flavonol pigments of *Cicer arietinum* leaves and stems showed the presence of kaempferol 3-(malonylglucoside) and kaempferol 3-(apiosylmalonylglucoside)

INTRODUCTION

In roots and aerial parts of *C. arietinum* L, the isoflavones biochanin A and formononetin predominantly occur as isoflavone 7-*O*-glucoside-6″-*O*-malonates [1]. The chickpea malonyl-CoA: isoflavone 7-*O*-glucoside-6″-*O*-malonyltransferase isolated and characterized from tissue [2] possesses a pronounced specificity for *iso*-flavone 7-*O*-glucosides though flavonol 7-*O*-glucosides were also malonated by the enzyme

Kaempferol, quercetin and isorhamnetin are the flavonol constituents of the aerial parts of chickpea plants, kaempferol-3-glucoside and kaempferol-3-apiosylglucoside have been found as the main conjugates [3]

In view of the rapidly increasing number of malonated flavonoid glucosides in plants [4–10] and the observed multiplicity of malonyltransferases in *C arietinum* [2] we have re-examined the flavonol pigments of chickpea We here report the identification of two malonated kaempferol glucosides in this plant

RESULTS

The phenolic constituents obtained from aerial parts of *C arietinum* plants by mild extraction were separated by HPLC (detection 350 nm) and assayed by UV spectroscopy using diagnostic reagents [11] Six compounds were thus shown to be flavonol 3-glucosides Using reference materials three minor constituents were identified as the 3-*O*-glucosides of kaempferol, quercetin and isorhamnetin, respectively A fourth compound was shown to be the previously described kaempferol 3-*O*-apiosylglucoside [3]

The two main, very polar flavonol compounds were isolated by polyamide column chromatography [1, 3] and preparative HPLC Both compounds migrated as anions during electrophoresis at pH 6 Upon mild alkaline hydrolysis one compound was converted to kaempferol 3-*O*-glucoside whereas the other polar constituent yielded kaempferol 3-*O*-apiosylglucoside Apiose was determined according to [12]. The acyl moiety of both polar flavonol constituents was identified as malonic acid by formation of *p*-coumaric acid upon reaction with *p*-hydroxybenzaldehyde [13] Acylated derivatives of quercetin and isorhamnetin could not be detected

In general, the most prominent flavonol constituents of chickpea are kaempferol 3-(malonylglucoside) [6] and kaempferol 3-(apiosylmalonylglucoside) In both compounds malonic acid is assumed to be attached to C-6 of glucose This represents the most widely occurring site of acyl attachment [5–9] which has also been shown for the isoflavone malonylglucosides of the chickpea plant [1]

EXPERIMENTAL

Polar phenolic compounds were extracted from aerial parts of 3-week-old chickpea plants with Me_2CO–MeOH according to [1] Polyamide CC of malonated flavonoid glucosides has been described [1] HPLC was conducted on RP-18 or RP-8 columns with a WATERS chromatograph attached to a photodiode array detector for recording UV-spectra A linear gradient of 30% B (acetonitrile) to 60% B in (A + B) in 25 min was applied Solvent A was 3% HOAC and the flow rate 1 ml/min Identity and purity of compounds were monitored by HPLC and TLC with solvents as in [6] Paper electrophoresis was performed in K-Pi buffer (p 1 M, pH 6) at 65 V cm Detection of malonic acid was as described [13] Determination of apiose was performed according to [12] UV spectroscopic data of the various flavonol derivatives were identical with published data [3, 6, 11]

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LUPIN ALKALOIDS FROM THE SEEDS OF *THERMOPSIS LUPINOIDES*

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Key Word Index—*Thermopsis lupinoides*, Leguminosae, seeds, lupin alkaloids, quinolizidine alkaloids, (+)-lupanine N_{16}-oxide, (+)-lupanine

Abstract—A new lupin alkaloid, (+)-lupanine *N*-oxide, was isolated from the seeds of *Thermopsis lupinoides* together with nine known alkaloids. The structure of the new compound was determined by spectroscopic methods and chemical transformations.

INTRODUCTION

Thermopsis lupinoides Link is a herbaceous plant containing a large amount of lupin alkaloids [1]. We have already reported the isolation of eight lupin alkaloids from the flowers, leaves, stems and roots of *T. lupinoides* [2]. In the present communication, we describe the isolation of a new alkaloid, (+)-lupanine *N*-oxide (**1**), from the seeds of *T. lupinoides* and the structural elucidation by spectroscopic methods and chemical transformations.

RESULTS AND DISCUSSION

From the 75% EtOH extract of the seeds of *T lupinoides*, a new alkaloid (**1**) was isolated in a yield of 0.005% of the fr. wt by repeated silica gel chromatography. We also isolated nine known lupin alkaloids, (+)-lupanine (**2**, main base), (−)-anagyrine, (−)-cytisine, (−)-*N*-methylcytisine, (−)-sparteine, (+)-17-oxolupanine, *N*-formylcytisine, baptifoline, and ammodendrine.

The in-beam HRMS spectrum of **1** indicated the molecular formula $C_{15}H_{24}N_2O_2$ (M^+, *m/z* 264.1838, calc. 264.1837). The fragment pattern of **1** was similar to that of (+)-lupanine (**2**). The IR spectrum showed the presence of an amido group (1640 cm^{-1}) and an *N*-oxide bond (930 cm^{-1}). In the ^{13}C NMR spectrum of **1** (Table 1), the signals of C-11, C-15 and C-17 were shifted downfield in the range, δ 7–14 compared to those of **2**. The up-field shifts, on the contrary, were observed in the signals of C-8, C-12 and C-14, because of the steric effects of axial *N*-oxide bond at *N*-16. These substituent effects of *N*-oxide group were also reported in other lupin alkaloids [3–5]. In the ^1H NMR spectrum of **1**, the signals in low field were assigned by decoupling experiments and comparison

Table 1 ^{13}C NMR chemical shifts of (+)-lupanine *N*-oxide (**1**) and (+)-lupanine (**2**) in $CDCl_3$

C	1	2	Δ(1–2)
2	172.1	171.3	+0.8
3	33.0	33.1	−0.1
4	19.4	19.7	−0.3
5	27.7	26.8	+0.9
6	61.8	60.9	+0.9
7	33.6	35.0	−1.4
8	22.7	27.4	−4.7
9	31.7	32.5	−0.8
10	47.0	46.8	+0.2
11	71.4	64.0	+7.4
12	27.7	33.6	−5.9
13	25.7	24.5	+1.2
14	20.3	25.3	−5.0
15	69.6	55.4	+14.2
17	65.2	52.9	+12.3