*Phytochemistry*, 1981, Vol. 20, pp. 530–531. © Pergamon Press Ltd. Printed in England. 0031-9422/81/030530-02 $02.00/0

# FLAVONOID GLYCOSIDES OF *KALANCHOE SPATHULATA*

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(*Revised received* 6 *June* 1980)

**Key Word Index**—*Kalanchoe spathulata*; Crassulaceae; flavonoids; quercetin 3-*O*-glucoside-7-*O*-rhamnoside; kaempferol 3-*O*-rhamnoside; patuletin 3,7-di-*O*-rhamnoside.

**Abstract**—A new glycoside, patuletin 3,7-di-*O*-rhamnoside, together with patuletin, quercetin, quercetin 3-*O*-glucoside-7-*O*-rhamnoside, kaempferol and kaempferol 3-*O*-rhamnoside were identified from leaves and flowers of *Kalanchoe spathulata*.

## INTRODUCTION

*Kalanchoe*, a genus of approximately one hundred species belonging to the Crassulaceae, is native to tropical Africa but has been naturalized throughout the tropics. Several of the species have been used medicinally [1, 2] and a number of species have been examined for their chemical constituents [3, 4]. The present paper documents the flavonoids of *K. spathulata* and is the first report of patuletin 3,7-di-*O*-rhamnoside in nature.

## RESULTS AND DISCUSSION

Quercetin and its 3-*O*-glucoside-7-*O*-rhamnoside, kaempferol and its 3-*O*-rhamnoside and patuletin and its 3,7-di-*O*-rhamnoside were identified from the leaves and flowers of *Kalanchoe spathulata*. The UV, MS, NMR and chromatographic data of known compounds are in agreement with literature values.

Chromatographic appearance, UV, $^1$H NMR and GLC data are in agreement for the identification of the new glycoside as patuletin 3,7-di-*O*-rhamnoside. The C-1″ proton for rhamnosyl was indicated at $\delta$ 4.53; however, resonance of two different rhamnosyl methyl groups appeared in the $^1$H NMR at $\delta$ 0.95 and at 1.56. The signal at 0.95 corresponded to the rhamnosyl at the C-3 position while the signal at 1.56 was a complex signal indicative of the sugar residue at the C-7 position [5]. NMR verified the absence of protons at C-5, C-3′, C-4′, C-3, C-7 and at the 6- or 8-positions. The $AlCl_3$/HCl spectral values indicated a 6-OR substitution pattern. MS, UV and $^1$H NMR [6] support the identification of the aglycone as patuletin (6-methoxy quercertin). MS analysis indicated a MW of 332 ($M^+$ 332, $C_{16}O_8H_{12}$ requires 332). The base ion (100%, 317) required the loss of a Me= from $M^+$ (70%, 332); fragment $A^+$ (24%, 182; required, 182) and fragment $B^+$ (19%, 137; required 137) agree with pathway I of Mabry and Markham [7]. The absence of a major ion $(M - Me - CO)^+$ (10%, 289; required, 289) is in agreement with the MS studies of patuletin by Bowie and Cameron [8]. The UV spectra agree with those of patuletin [6].

Acid hydrolysis of the glycoside (2N HCl, 45 min) gave patuletin and rhamnose. Partial hydrolysis (0.5 N HCl, 5 min, 27 and 100°) produced two chromatographic spots (15% HOAc) in addition to the parent compound and patuletin. Under UV one of these ($R_f$ 0.48) appeared purple while the other ($R_f$ 0.07) appeared yellow. From comparisons with known quercetin derivatives, 3- or 7-*O*-rhamnosides [9, 10], the former represents the 3-*O*-rhamnoside and the latter the 7-*O*-rhamnoside. Hence, the glycoside was identified as patuletin 3,7-di-*O*-rhamnoside.

## EXPERIMENTAL

Plant material was collected from 'Bridal Path', Kasuali, Himachal Pradesh in September; a voucher specimen was deposited in the Herbarium at Panjab University. Dried leaves and flowers (1.5 kg) were Soxhlet-extracted with (1) petrol (60–80°) and (2) EtOH (100%). The conc EtOH extract (57.8 g) was taken up in $H_2O$ and the aq. mixture extracted with $Et_2O$ (8 × 100 ml) and then EtOAc (10 × 150 ml). The EtOAc concentrate (14 g) was mixed with $H_2O$ (250 ml), refrigerated overnight, the upper layer decanted and the inorganic residue discarded. Neutral $Pb(Ac)_2$ (saturated soln) was added to the decanted material and the ppt. collected by filtration. The orange-yellow lead complex was suspended in MeOH and a continuous stream of $H_2S$ passed through the soln; the ppt. was discarded. The filtrate was taken to dryness *in vacuo*, resuspended in $H_2O$ and extracted with EtOAc (12 × 50 ml). The conc EtOAc extract (6.25 g) was run on 1D PC (BAW, 4:1:5); the resulting chromatographic bands were individually rechromatographed (BAW, 4:1:1). The flavonoids were eluted with hot MeOH and crystallized. Identification was based on UV and NMR analysis of the glycosides with subsequent UV, MS and co-chromatography of the aglycones. $R_f$ values were determined on Whatman No. 1 (ascending) and sugars were identified by GLC.

*Patuletin-3,7-di-O-rhamnoside.* Mp 230–240°; $R_f$ (TBA, 0.46; 15% HOAc, 0.81; BAW, 0.50), UVnm: MeOH 373, 266; NaOMe

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401, 273; $AlCl_3$ 443, 362 (minor), 280; $AlCl_3$/HCl 401 (sh), 354, 276, NaOAc 394, 269; NaOAc $H_3BO_3$ 372, 264; $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.50 (*d*, $J$ = 2 Hz, H-6'); 7.33 (*d*, $J$ = 2 Hz, H-2'); 6.78 (*d*, $J$ = 2 Hz, H-5'); 6.60 (*m*, H-8 or H-6); 4.53 (*m*, rhamnosyl C-1" proton); 3.12 (*s*, OMe); 0.95 (*d*, $J$ = 2 Hz, rhamnosyl Me); 1.56 (complex signal rhamnosyl Me); and 12.5 for resonating proton (free C-5-OH group).

Patuletin obtained on acid hydrolysis had: mp 265–270°; UVnm: MeOH 374, 346sh, 273, 260; NaOMe decomposition; $AlCl_3$ 460, 275, 260sh; $AlCl_3$/HCl 431(sh), 363, 266; NaOAc 410, 332, 278; NaOAc/$H_3BO_3$ 392, 272sh, 264; $^1H$ NMR spectrum showed peaks at $\delta$ 7.83 (*s*, H-6'); 7.62 (*s*, H-2'); 7.20 (*dd*, $J$ = 4 and 4 Hz, H-5'); 6.50 (*d*, $J$ = 3 Hz, H-8 or H-6); 3.15 (*s*, OMe); and at low field at 23.5 (C-5-OH proton). MS gave a molecular ion peak at *m/e* 332 ($M^+$), and other characteristic peaks at *m/e* 182 (24%) (A-ring), 137 (19%) (B-ring), 317 (100%) (base ion); PC $R_f$ 0.03 (15% HOAc), 0.61 (BAW). The sugar fraction was identified by GLC (3% SE 52, 3% OV-1, 3% Poly A-101 A) as rhamnose.

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