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CHROMENE GLYCOSIDES FROM *AGERATINA ALTISSIMA*

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Key Word Index –*Ageratina altissima*, Asteraceae, chromene glycosides, structure elucidation

Abstract—This is the first report of hydrophilic chromene conjugates from species of the Asteraceae Leaves from *Ageratina altissima* yielded two novel glycosides that were both based on the aglycone 2-hydroxymethyl-2-methyl-6-acetylchromene Whereas β-glucose was identified as the sugar moiety for both glycosides one compound was further distinguished by the presence of 3-hydroxy-3-methylglutaric acid, as the ester, bound to C-6 of the glucose residue

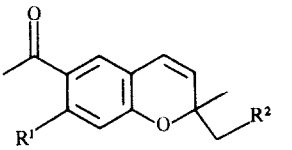
INTRODUCTION

2,2-Dimethylchromenes are characteristic metabolities of many species of the Asteraceae primarily from the tribes Astereae, Eupatorieae, Heliantheae, Inuleae and Senecioneae [1, 2] All of the 50 or so naturally occurring chromenes from the Asteraceae are lipophilic compounds No hydrophilic chromene conjugates have been reported from this family to date In a continuation of our studies on chromenes and benzofurans from the Asteraceae, we have analysed leaves of *Ageratina altissima* (tribe Eupatorieae) and found them to contain two novel chromene glycosides besides several lipophilic chromenes previously reported for this species [3] To our knowledge this is the first report on chromene glycosides from the Asteraceae. The structure elucidation of these new compounds is described

RESULTS AND DISCUSSION

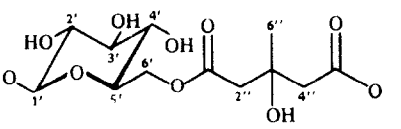
A methanol extract from the leaves of flowering plants of *Ageratina altissima* (L) R M King and H Robinson was found to contain the well known lipophilic chromene derivatives encecalin, demethylencecalin and demethoxyencecalin (**1**–**3**) as previously reported [3, 4] Acidic hydrolysis of the water-soluble parts of the extract liberated a further chromene derivative which was identified as 2-hydroxymethyl-2-methyl-6-acetylchromene (aglycone of **4** and **5**) by GC/MS and by direct comparison with an authentic sample [5] Since alkaline hydrolysis did not result in the liberation of the aglycone, it was assumed that the nature of the chromene conjugate was glycosidic The water-soluble portion of the methanol extract was subsequently separated by various chromatographic procedures (involving CC on polyamide, silica gel and Sephadex LH-20) into fractions followed by acidic hydrolysis of a aliquot of each fraction and subsequent HPLC analysis for the presence of the liberated aglycone (this served as a method of finding the fractions containing the chromene conjugates). This led to the isolation of the two glycosides (**4** and **5**) that both exhibited the same chromene aglycone

The ^{1}H NMR spectrum of compound **4** indicated the presence of 2-hydroxymethyl-2-methyl-6-acetylchromene as one part of the molecule as shown by direct comparison with the ^{1}H NMR spectrum of an authentic sample [5] The ^{1}H NMR spectrum further more showed the presence of a pyranose sugar moiety The chemical shift of the proton at C-1′ (δ 4 328) and the coupling constant J(1′–2′) of 7 7 Hz indicated that the glycosidic bond was at C-1′ and that the sugar had a β-configuration This moiety could only be substituted at C-14 of the chromene The M_r of **4** of 524 (FABMS) indicated that a further residue was present in the molecule This residue had to be esterified at C-6′ of the pyranose as indicated by the chemical shifts of the two protons H-6′A and H-6′B



	R^1	R^2
1	H	H
2	OH	H
3	OMe	H
4	H	A
5	H	B

A =



B = β-glc

(δ 4 456 and 4.220, respectively). Signals in the ^{1}H NMR spectrum of **4** that could be ascribed to this residue included two isolated spin systems (one centred at δ 2.654 and a second at δ 2.590 and 2 460, respectively) and a singlet of a methyl group at δ 1.364. From the MS and ^{1}H NMR data 3-hydroxy-3-methylglutaric acid was a likely candidate for this residue. Acidic hydrolysis followed by GC/MS analysis of the trimethylsilyl derivatives of the hydrolysis products and comparison with a commercially available sample unambigously identified 3-hydroxy-3-methylglutaric acid as the esterifying acid It further corroborated the nature of the chromene aglycone and the presence of glucose as the sugar moiety was unambiguously confirmed.

A further chromene conjugate (**5**) was isolated in smaller amounts, and exhibited a M_r of 380 (FABMS). Its structure followed from a comparison of its ^{1}H NMR spectrum with that of **4** and from GC-MS analysis of its hydrolysis products.

The small amounts of **4** and **5** isolated precluded the determination of the absolute stereochemistry of these compounds.

EXPERIMENTAL

Plants of *Ageratina altissima* were grown in the experimental garden of the Institut fur Pharmazeutische Biologie A voucher specimen is on file Leaves of flowering plants were harvested, immediately freeze-dried and extracted with 70% aq MeOH at room temp. The MeOH extract was taken to dryness and partitioned between CH_2Cl_2 and H_2O The H_2O fraction was concd by freeze-drying and separated by CC on Polyamide SC-6 Various mixtures of H_2O and MeOH were used as eluents An aliquot of each fraction obtained was hydrolysed in 1 M HCl for 1 hr at 90°. After dilution with MeOH (1 1), 20 μl were injected and analysed by HPLC for the presence of 2-hydroxymethyl-2-methyl-6-acetylchromene (available as reference sample) The HPLC apparatus, the separation conditions and the origin of the reference sample have recently been described [5] The fraction containing the chromene glycosides was further separated by CC on silica gel with a mixture of EtOAc–HCOOH–HOAc–methylethylketone–H_2O (50 7 3 30 10) as eluent and by CC on Sephadex LH-20 with MeOH as eluent Fractionation was again accompanied by hydrolysis and HPLC analysis as described above. Final purification of **4** and **5** was achieved by prep TLC on premade silica gel plates with the solvent system described above followed by CC on Sephadex LH-20. Chromenes **1–3** were identified by GC/MS analysis of the CH_2Cl_2 fraction The GC/MS apparatus and the separation conditions have also recently been described [5]

^{1}H NMR spectra were recorded at 300 or 400 MHz respectively All 1D and 2D spectra were obtained using the standard Bruker software Chemical shifts are relative to TMS and coupling constant are in Hz Negative ion FAB mass spectra were measured on a Finnigan MAT 8430 mass spectrometer with glycerol as matrix For GC/MS conditions see [5]

Compound **4** ^{1}H NMR (CD_3OD) δ 7.831 (*d*, *d*, H-7, *J* (7–5) 2 1, *J* (7–8) 8 5), 7 725 (*d*, H-5), 6 856 (*d*, H-8), 6.566 (*d*, H-4), *J* (3–4) 10 0), 5.886 (*d*, H-3), 4 456 (*d*, *d*, H-6′A, *J* (6′A–5′) 2, *J* (6′A–6′B) 11 9), 4.328 (*d*, H-1′, *J* (1′–2′) 7 7), 4 220 (*d*, d, H-6′B, *J* (6′B–5′) 5 7), 3 960 (*d*, H-14A, *J* (14A–14B) 11), 3.690 (*d*, H-14B), 3 50–3 28 (*m*, H-3′, H-4′), 3 46 (*m*, H-5′), 3 20 [*d*, *d*, H-2′, *J* (2′–3′) 9.7], 2 654 (AB centre, H-4″ A, B), 2.590 (*d*, 11-2″A, *J* (2″A–2″B) 15 4], 2 575 (*s*, 6-COMe), 2 460 (*d*, H-2″B), 1 486 (*s*, H-13), 1 364 (*s*, H-6″) FABMS *m/z* 523 $[M-H]^-$, 217 [chromene aglycone – H].

Compound **5**. ^{1}H NMR (CD OD) δ 7 836 (*d*, *d*, H-7, *J* (7–5) 2 1, *J* (7 – 8) 8.7], 7 729 (*d*, H-5), 6 857 (*d*, H-8), 6 573 [*d*, H-4, *J* (3–4) 10.0], 5 913 (*d*, H-3), 4.325 [*d*, H-1′, *J* (1′–2′) 7.6], 4 057 [*d*, H–14A, *J*, (14A–14B) 10.2], 3.9–3 2 (*m*, H-14B, H-2′ to H-6′), 2 574 (*s*, 6-COMe), 1 498 (*s*, H-13) FABMS *m/z* 379 $[M-H]^-$, 217 [chromene aglycone – H]$^-$

3-Hydroxy-3-methylglutaric acid as trimethylsilyl derivative. GC/MS *m/z* (rel int.): 363 (2), 273 (4), 247 (10), 231 (6), 204 (2), 199 (7), 183 (2), 147 (27), 115 (14), 109 (8), 73 (100) The spectrum and retention index (RI = 1610) were identical to those of a commercially available sample (Sigma, F R G)

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