Kaempferol-3-O-β-*rhamninoside* (**2**). A yellow powder, UV λ_{max}^{MeOH} nm 266 350 (log ε 4.3, 4.2, respectively), $[\alpha]_D^{22}$ −42.7° (MeOH, *c* 0.99), Mg–HCl test: positive, MS (peracetate): *m/z* 791 [(Gal-Rha-Rha)Ac_8]$^+$, 503 [(Rha-Rha)Ac_5]$^+$ and 273 [(Rha)-Ac_3]$^+$. Methylation analysis of the sugar moiety was carried out according to the method of refs [3, 4].

Rhamnocitrin-3-O-β-*rhamninoside* (**3**). A yellow powder, Mg–HCl test: positive, $[\alpha]_D^{13}$ −45.1° (MeOH, *c* 0.71).

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A BENZOFURAN FROM *AGERATUM HOUSTONIANUM*

REGINA SIEBERTZ, PETER PROKSCH,* VICTOR WRAY† and LUDGER WITTE

Institut für Pharmazeutische Biologie, der TU Braunschweig, Mendelssohnstrasse 1, D-3300 Braunschweig, F.R.G.; †Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, F.R.G.

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Key Word Index—*Ageratum houstonianum*; Asteraceae; benzofuran; structure elucidation.

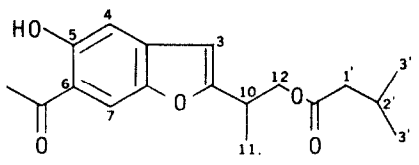
Abstract—A new benzofuran was isolated from roots of *Ageratum houstonianum* that was characterized by the presence of the acetyl substituent at C-6 and not at C-5 as usually encountered. The structure elucidation is described.

INTRODUCTION

Ageratum houstonianum Mill. (tribe Eupatorieae) has attracted considerable attention due to the presence of the chromene derivatives precocene I and II that act as antijuvenile hormones against a plethora of susceptible insects [1]. Whereas the leaves and flowering heads of *A. houstonianum* are the favoured organs of precocene accumulation, the roots are distinguished by the accumulation of benzofuran derivatives [2]. Recently [2] we have reported on the structure elucidation of several benzofurans from roots of *A. houstonianum* that were distinguished by the presence of the acetyl substituent at C-6 of the aromatic ring and not at C-5 as previously reported [4] and usually encountered with benzofurans from species of the Asteraceae [5]. In continuation of our studies on *A. houstonianum*, we have now isolated a further unusual benzofuran derivative from roots and wish to report on the structure elucidation.

RESULTS AND DISCUSSION

The new benzofuran (**1**) exhibited a ^{1}H NMR spectrum typical for a acetylbenzofuran as indicated by the signal of



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*Author to whom correspondence should be addressed.

the MeCO group (2.677 ppm), the furan proton (δ 6.416) and the signals of two aromatic protons (δ 7.005 and 7.772) that could be ascribed to H-4 and H-7 respectively. It furthermore showed the signal of a OH-group which had to be *ortho* to the acetyl group as indicated by the sharp singlet at δ 12.155 caused by the formation of a stable H-bond. The aromatic proton responsible for the signal at δ 7.772 also had to be *ortho* to the MeCO group. The chemical shifts of the two aromatic protons thus were compatible both with 5-acetyl-6-hydroxy-benzofuran and with its 6-acetyl-5-hydroxy-isomer. The signal of the furan proton (δ 6.416) and the downfield signal of one aromatic proton (δ 7.772), however, were broadened due to long range coupling (confirmed by a long-range ^{1}H 2D spectrum) between H-3 and H-7 (3, 6). The unambiguous assignment of H-4 and H-7 followed from a ^{1}H NOE difference spectrum as the signal of H-4 showed a NOE upon irradiation of H-3. As H-7 showed a NOE upon irradiation of the methyl of the acetyl group the position of the latter was identified. Further signals in the ^{1}H NMR spectrum originated from the side chain present at C-2 of the furan ring and from the esterified dihydroenecioic acid. Thus the signal of the Me group (C-11) at δ 1.395 was a doublet from coupling with the proton at C-10 (*dd* 3.317) and this showed further coupling to the protons at C-12 (*dd* δ 4.365 and *dd* 4.278) as was evident from the COSY-spectrum. The nature of the esterifying acid also followed from the ^{1}H NMR data and COSY-spectrum. Thus the signal of the two protons at C-1′ was a doublet from coupling with the proton at C-2′ which in turn gave rise to a multiplet due to coupling with the geminal methyl groups C-3′a and C-3′b. The latter appeared as overlapping doublets at 0.902 ppm. The mass spectrum of **1** confirmed the structure and showed the molecular ion at *m/z* 318. The base peak was observed at *m/z* 216 and corresponded to the fragment $[M-HOOCCH_2CH(Me)_2]^+$ which in turn lost 15 mass units to give rise to the second largest fragment at *m/z* 201.

The basic structure of this new benzofuran thus corresponds to the benzofurans isolated previously by us [3] from roots of *A. houstonianum* that were likewise distinguished by the presence of the acetyl substituent at C-6 of the aromatic ring. Benzofurans with this unusual skeleton seem to be very rare within the Asteraceae and are known outside of *Ageratum* only for a few species of *Baccharis* (tribe Astereae) and *Leibnitzia* (tribe Mutisieae) [5 and refs therein]. The spectroscopic features characteristic for a 6-acetylbenzofuran on comparison to those of a 5-acetylbenzofuran, however, are rather inconspicuous and only become obvious when NOE or COSY experiments are performed. Thus the possibility remains that a substantially larger number of these unusual benzofurans has been isolated in the past but incorrectly identified as has happened for several compounds from *A. houstonianum* [3, 4].

EXPERIMENTAL

Achenes from *A. houstonianum* var. *Blaue Donau* were commercially available from Walz Qualitätssamen (Stuttgart, F.R.G.). Plants were grown in a greenhouse in wet sand and fertilized once a week with Polycrescal, Schering AG (Berlin, F.R.G.) at a concn of 1 g/l H_2O until flowering. Immediately after harvesting roots were extracted with Me_2CO. The crude extract was taken to dryness, redissolved in CH_2Cl_2 and separated on a silica gel column with CH_2Cl_2–MeOH (99:1) as eluent. Fractions of 20 ml were collected and monitored on TLC (silica gel, same solvent system). The benzofurans were detected by their orange to yellow fluorescence under UV (366 nm). Similar fractions were combined, taken to dryness, redissolved in MeOH and chromatographed on a Sephadex LH-20 column with MeOH as eluent. Final purification of **1** was achieved by prep. TLC (for parameters see above) followed again by CC on Sephadex LH-20.

^{1}H NMR spectra were recorded at 300 MHz. Chemical shifts are relative to TMS and coupling constants are in Hz. ^{1}H NMR ($CDCl_3$) δ 12.155 (*s*, 5-OH), 7.72 (*s*, H-7; small couplings *J* (7–4) and *J* (7–3) detected in long-range COSY spectrum), 7.005 (*s*; H-4), 6.416 (*s*; H-3), 4.365 [*d*, *d*; H-12A; *J*(12A–12B) 10.9, *J*(12A–10) 6.7], 4.278 [*d*, *d*; H-12B; *J*(12B–10) 6.1], 3.317 (*m*; H-10), 2.677 (*s*, 6–COMe), 2.172 (*d*, H-1′; *J*(1′–2′) 7.0), 2.057 (*m*; H-2′), 1.395 [*d*, H-11; *J*(11–10) 7.0], 0.902 [*d*, H-3′a, H-3′b; *J*(3′a, 3′b - 2′) 6.6].

The mass spectrum was recorded by GC-MS at 70 eV in the EI mode. MS (*m/z*, rel. int.): 318 (3), 216 (100), 201 (74), 160 (5), 85 (3), 57 (8), 43 (9).

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