Phytochemistry, Vol. 26, No. 11, pp. 3055–3057, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

BENZOFURAN DERIVATIVES FROM *AGERATUM HOUSTONIANUM*

MATTHIAS BREUER, HERBERT BUDZIKIEWICZ, REGINA SIEBERTZ* and PETER PROKSCH*†

Institut für Organische Chemie der Universität zu Köln, Greinstrasse 4, D-5000 Köln 41, F.R.G.; *Institut für Pharmazeutische Biologie der TU Braunschweig, Mendelssohnstrasse 1, D-3300 Braunschweig, F.R.G.

(*Received* 26 *February* 1987)

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

Abstract—The structures of several novel benzofurans from the roots of *Ageratum houstonianum* have been elucidated. One of them is apparently identical with the previously isolated ageratone the structure of which has now been revised.

INTRODUCTION

From roots of *Ageratum houstonianum* Mill. var. Blaue Donau several novel benzofuran derivatives were isolated which are of phytochemical and taxonomic interest due to their unusual substitution pattern: 6-acetyl-5-hydroxy-2-isopropenyl-benzo[b]furan (**1**) and its hydroxy (**2**) and acetoxy (**3**) derivatives as well as the 'dimeric' representatives discussed below.

RESULTS AND DISCUSSION

The mass spectrum of (**1**) exhibits a molecular ion *m/z* 216; the fragmentation pattern (loss of Me· from M^+·, *m/z* 201, followed by that of CO, *m/z* 173, for further ions see Experimental) is very similar to that of euparin, i.e. 5-acetyl-6-hydroxy-2-isopropenyl-benzo[*b*]furan (**4**). The ^{1}H NMR spectrum (see Table 1) clearly shows the presence of the Me of the acetyl group, the isopropenyl residue (Me signal at 2.11 ppm split to a *dd* by coupling with the methylene protones at 5.28 and 5.84 ppm as shown by double resonance experiments) and 3 *s* in the aromatic region which are compatible only with a 2,5,6- or 3,5,6-substitution of the benzo[b]furan nucleus. The acetyl and the hydroxy group must be in an *O*-position to each other (C-5, C-6) which allows the formation of a stable H bridge responsible for the sharp *s* at 12.13 ppm. Two of the three aromatic *s* (6.56 and 7.78 ppm) are broadened by long range coupling (confirmed by double resonance experiments), the so-called W-effect which is possible only between H-atoms at C-3 and C-7. Hence, the furan ring must carry the isopropenyl group in 2-position. As the signal observed at lowest field is the one to be attributed to the H adjacent to the acetyl group the latter has to be located at C-6 since the signal at 7.78 ppm is the one which has been broadened and, therefore, to be assigned to the H at C-7. This argument can be corroborated by the ^{1}H NMR spectrum of euparin [1–3] (**4**) where the position of the substituents at C-5 and-6 is reversed as compared with **1**. Here the signal at higher field (6.96 ppm) stemming from the H neighbouring the OH group is the broadened one.

The ^{1}H NMR spectra of **2** (mass spectrum M^+· *m/z* 232, $[M - \cdot Me]^+$ *m/z* 217) and of **3** (mass spectrum: M^+· *m/z* 274, $[M - Me]^+$ *m/z* 259, $[M - C_2H_2O]^+$ *m/z* 232, $[M - \cdot Me - C_2H_2O]^+$ *m/z* 217) differ from that of **1** only in the aliphatic region demonstrating the presence of a CH_2OH and a CH_2OCOMe group, respectively instead of Me. In a short communication [4] Anthonsen and Chantharasakul described the isolation of ageratone from *Ageratum houstonianum* Mill. for which they suggested structure (**5**), based mainly on spectral evidence (IR, mass and especially ^{1}H NMR). The substitution pattern including the substituents of C-5 and C-6 (acetyl and hydroxyl group) was based only on analogy with that of euparin (**4**) as the NMR spectrometers available in 1970 did not provide the resolving power necessary for detailed analysis. For a justification they pointed out the close taxonomic relationship between *Ageratum* and *Eupatorium*. The marked differences in the UV spectra of euparin [2, 3] and ageratone [4] they ascribed 'to differences around the bond connecting the isopropenyl group to the furan nucleus'.

As can be seen from Table 2 the UV spectra of euparin (**4**) and its isomer differ markedly while those of **1** and **3** correspond closely which indicates that the UV absorptions are determined by the substitution pattern rather than by modifications in the isopropenyl residue. The ^{1}H NMR spectra of **3** (Table 1) and ageratone [4] differ only by a few hundredths of a ppm which is well within the accuracy of measurements (and thus that of ageratone differs to a larger degree from that of **4** than from that of **1**; see Table 1) The melting points are identical. This and the isolation from the same plant species would suggest that ageratone actually has structure **3**. The published UV spectrum [4], however, is shifted by 20 nm as compared with that of **3**, but it has definitely not the shape of that of **4**. The best suggestion is that ageratone does have structure **3** and that there has been an error in the assignment of the UV wavelengths.

The structure of 4-acetoxymethyl-7-acetyl-4-[2′-(6′-acetyl-5′-hydroxy-benzo[b]furanyl)]-6-hydroxy-1-methylene-1,2,3,4-tetrahydro-dibenzo[b, d]furan (**6**) follows from the mass and NMR spectral data: M^+· (*m/z* 488, $C_{28}H_{24}O_8$ by exact mass measurement) loses the CH_2OCOMe-group (*m/z* 415, $C_{25}H_{19}O_6$, base peak). The only other fragment of importance is *m/z* 43 [$MeCO^+$].

† Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data of 1–4 (ppm rel. to ($CHCl_3$ = 7.24 ppm)

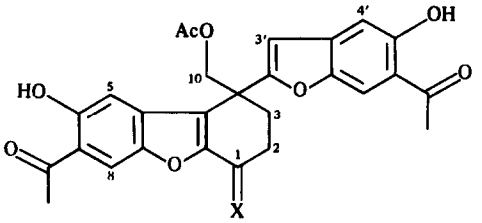
	1	2	3	4	
5-OH	12.13	12.25	12.10	12.50	*s*
H-7	7.78	7.79	7.79	6.96	*s br**
H-4	7.01	7.03	7.03	7.89	*s*
H-3	6.56	6.69	6.64	6.53	*s br**
H-1'a	5.84	5.99	6.07	5.74	*m*†
H-1'b	5.28	5.58	5.60	5.17	*m*†
CH_2R	2.11	4.52	4.93	2.08	*dd*† (*J* = 1.3/0.8 Hz)
COMe	2.67	2.67	2.67	2.67	*s*
OCOMe	—	—	2.10		*s*

*Irradiation at H-7 gives a sharp *s* for H-3.

†Irradiation at the CH_2R-signal results in a simplification of the signals for H-1'a and H-1'b (*d*, *J* = 1.3 Hz).

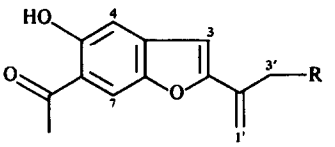
The substitution pattern of the benzofuran ring has been deduced from the ^{1}H NMR spectrum (see Table 2) in the same way as described for **1** [see especially the ω-coupling between the signals at 7.73 (H-7') and 6.41 ppm (H-3')]. The two remaining signals in the aromatic region show only a minute *p*-coupling and, therefore, have to be attributed to H-5 and H-8. Obviously, due to the absence of a proton at the β-position of the furan ring, C-4a, the signal neighbouring the substituent at C-7 cannot be identified by a ω-coupling. The UV spectrum (see Table 2), however, confirms the position of the acetyl and hydroxyl group. The structure of the cyclohexene ring follows from the decoupling experiments: Irradiation at the *m* at 2.6 ppm results in a now sharp *d* for the olefinic protons as well as in a simplification of the two *ddd* systems at 2.17 and 2.40 ppm (now *dd*). The fact that the protons responsible for the two *ddd* systems are diastereotopic requires a neighbouring chiral quaternary C-atom. Thus the sequence $H_2C{=}CH_2{-}CH_2{-}C{-}$ has been established. An exchange of the substituents at C-1 and C-4 would also be compatible with the NMR-spectral argumentation, but only **6** can be ontained by condensation of two units of **1**, and hence the arrangement depicted in **6** is the more likely.

Owing to the small amounts of material isolated for two additional compounds, only structural suggestions can be made. One of them as shown by exact mass measurements has an elemental composition of $C_{30}H_{28}O_{11}$ ($M^{+\cdot}$ *m/z* 564). From the ^{1}H NMR spectrum the same skeleton as for **6** can be deduced (see Table 2). The olefinic signals have disappeared and that of C-2-H has been shifted to a somewhat higher field (2.05 ppm) being not any more allylic. An additional acetoxymethyl signal and an AB-system of a CH_2OH group would account for the substitution at C-1. The broad *s* at 2.73 can be attributed to an OH-group since it disappears upon irradiation of the H_2O signal at 1.54 ppm. The mass spectrum corroborates the nature of the substituents: M^+ loses MeCOOH (*m/z* 504) as well as a CH_2OCOMe group (*m/z* 491; cf **6**). The ion *m/z* 491 in turn loses MeCOOH (*m/z* 431) and subsequently H_2O (*m/z* 413). Taking into

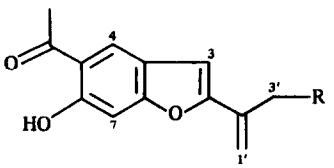


6 X = CH_2

7 X = C_9H_2OAc(OH)



1 R = H
2 R = OH
3 R = OAc



4 R = H
5 R = OAc

Table 2. ^{1}H NMR data of **6–8** (cf. Table 1)

	6	7	8	
6-OH‡‡	12.10	12.10	12.10	*s*
5′-OH‡‡	12.06	12.03	12.00	*s*
H-8	7.82	7.75	7.85	*s*†
H-7′	7.73	7.59	7.74	*s br**
H-4′	6.96	6.93	6.99	*s**
H-5	6.92	7.09	6.90	*s*†
H-3′	6.41	6.28	6.48	d* (J = 0.9 Hz)
H-9a	5.78‡	4.59‖	4.05§	
H-9b	5.23‡	4.30‖	3.84§	
H-10a	4.71‖	4.72‖	4.67¶	
H-10b	4.70‖	4.70‖	4.64¶	
9-OH	—	2.73**	2.94**	*s br'*
6′-COMe‡‡	2.67	2.68	2.66	*s*
7-COMe‡‡	2.63	2.65	2.63	*s*
H-1	—	—	2.2	*m*
H-2a, b	2.6	2.05	2.2	*m*
H-3a	2.40††	2.35	2.03	*m*
9-OCOMe	—	2.06‡‡	—	*s*
10-OCOMe	1.97	1.99‡‡	1.97	*s*

*Irradiation at H-7′ results in a smaller line width of the signal for H-4′ and to a sharp *s* for H-3′.

†Irradiation at H-5 results in a smaller line width of the signal for H-8.

‡*d br* sharpened by irradiation at H-2.

§*dd*, ^2J(H-9a, H-9b) 11.3 Hz, ^3J(H-9a, H-1) 4.9 Hz, ^3J(H-9b, H-1) 8 Hz.

‖AB system with ^2J 11.4 Hz.

¶AB system with ^2J 11.3 Hz.

**Disappears upon irradiation of the H_2O signal at 1.54 ppm.

††*ddd*, ^2J(H-3a, H-3b): 13.8 Hz; ^3J(H-3a, H-2) 8.4 Hz and 4.2 Hz; ^3J(H-3b, H-2) 8.5 Hz and 4.3 Hz.

‡‡Assignment may be reversed.

UV-data of the compounds **1–8** (λ nm, log ε, solvent C_2H_5OH).

1: 220§§, 305 (4.20); **2**: 220§, 310 (3.76); **3**: 220§§, 305 (4.11); **4**: 238 (sh), 262, 289, 300 (sh), 355 238 (sh) 263 (4.55), 289 (4.18), 302 (4.06), 360 (3.78) (lit.[4]) "**5**" 240 (3.67), 325 (4.00); **6**: 220§§, 307; **7**: 220§§, 300; **8**: 220§§, 300.

§§Approximately due to the beginning absorption of ethanol.

account the co-occurence with **6**, these data are compatible with structure **7**. The other compound (**8**) has an elemental composition of $C_{28}H_{26}O_{10}$ ($M^{+\cdot}$ *m/z* 522). An abundant $[M - CH_2OCOCH_3]^+$ ion (*m/z* 449) is also present in this mass spectrum. The ^{1}H NMR data (Table 2) demonstrate the close relationship between **7** and **8**, the main difference being that C-1 carries an H which couples with the C-9-CH_2-group. A problem is the nature of the tenth oxygen atom (possibly a peroxy-group).

EXPERIMENTAL

Plant material and isolation. Achenes of *A. houstonianum* var. Blaue Donau were commercially available from Walz Qualitätssamen, Stuttgart. A specimen has been deposited in the herbarium of the Institut für Pharmazeutische Biologie, Braunschweig. Plants were grown on wet sand and fertilized once a week with Polycrescal, Schering AG, Berlin, at a concentration of 1 g/l H_2O. Six weeks after germination (shortly before flowering) the plants were harvested. Roots were exhaustively extracted with Me_2CO. The crude extract was taken to dryness redissolved in CH_2Cl_2 and separated on a Sigel column with CH_2Cl_2–MeOH 99:1 as eluent. Fractions of 20 ml were collected and monitored on TLC (Sigel, same solvent system). 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 the benzofurans was achieved by recrystallization from MeOH (3 as yellow needles) or by prep. TLC on Sigel with CH_2Cl_2/MeOH (99:1) as solvent.

6-*Acetyl-5-hydroxy-2-isopropenyl-benzo*[*b*] *furan* (**1**). UV: see Table 2. ^{1}H NMR. See Table 1. Mass spectrum *m/z* (rel. int.) 217 (7), 216 (49), 202 (12), 201 (100), 173 (9).

6-*Acetyl-5-hydroxy-2-(1-hydroxymethylvinyl)-benzo*[*b*] *furan* (**2**). UV: see Table 2. ^{1}H NMR: see Table 1. Mass spectrum *m/z* (rel. int.) 233 (7), 232 (50), 218 (13), 217 (100), 203 (4), 159 (9).

6-*Acetyl-5-hydroxy-2-(1-acetoxymethylvinyl)-benzo*[*b*] *furan* (**3**). Mp 123–125°. UV: see Table 2. ^{1}H NMR: see Table 1. Mass spectrum *m/z* (rel. int.) 275 (9), 274 (52), 260 (14), 259 (100), 232 (16), 217 (11), 216 (7), 203 (8), 201 (11), 200 (9), 159 (4), 43 (72).

Euparin (**4**). Mass spectrum *m/z* (rel. int.) 217 (11), 216 (73), 203 (5), 202 (13), 201 (100), 198 (9), 174 (4), 173 (18), 115 (10), 101 (5), 100 (5).

4-*Acetoxymethyl-7-acetyl-4-*[2′-(6′-*acetyl-5′-hydroxy-benzo* [*b*] *furanyl*)]-6-*hydroxy-1-methylene-1,2,3,4-tetrahydrodibenzo* [*b, d*] *furan* (**6**). UV: see Table 2 ^{1}H NMR see Table 2. Mass spectrum *m/z* (rel. int.) 488. 1462 (4) (calcd. 488.1471 for $C_{28}H_{24}O_8$), 417 (4), 416 (16), 415.1191 (55) (calcd) for 415.1200 $C_{25}H_{19}O_6$), 189 (6), 43 (100).

1,4-*Bis-acetoxymethyl-7-acetyl-4-*[2′-(6-*acetyl-5′-hydroxy-benzo* [*b*]*furanyl*)]-6,7-*dihydroxy-1,2,3,4-tetrahydrodibenzo* [*b, d*] *furan* (**7**). UV: see Table 2. ^{1}H NMR: see Table 2. Mass spectrum *m/z* (rel. int.) 564 (7), 505 (9), 492 (14), 491 (48), 433 (14), 432 (18), 431 (91), 414 (26), 413 (88), 404 (7), 403 (27), 402 (10), 401 (16), 399 (5), 388 (9), 386 (11), 384 (5), 361 (8), 359 (13), 255 (13), 190 (8), 189 (45), 177 (6), 60 (35), 43 (100). Exact mass: FAB $[M + H]^+$ *m/z* 565.1705 (calcd 565.1710 for $C_{30}H_{29}O_{10}$).

Compound **8**. UV: see Table 2. ^{1}H NMR: see Table 2. Mass spectrum *m/z* (rel. int.) 522 (5), 450 (9), 449 (19), 419 (8), 418 (9), 417 (8), 226 (3), 225 (9), 203 (13), 201 (6), 199 (7), 189 (14), 177 (5), 43 (100), Exact mass: FAB $[M + H]^+$ *m/z* 523.1629 (calcd 523.1604 for $C_{28}H_{27}O_{10}$).

Acknowledgement—This work was supported by a grant of the DFG awarded to P. P.

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