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CHROMENES FROM *AGERATINA RIPARIA*

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Key Word Index—*Ageratina riparia*; Compositae; new chromenes.

Abstract—The reinvestigation of the roots and aerial parts of *Ageratina riparia* afforded ten new chromenes, their structures being determined by spectral data and chemical transformations.

INTRODUCTION

The chemistry of the large genus *Ageratina* (Compositae, tribe Eupatorieae, subtribe Ageratinae) has been studied by many groups [1–9] and several chromenes [1–5], which are widespread in the genus, have been isolated. A previous study [10, 11] of *A. riparia* (Regel) King et Rob., a widespread weed, has shown it to contain a number of chromenes. We now wish to report the isolation of ten new chromenes from *A. riparia* collected in Hawaii.

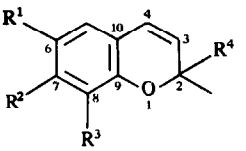
RESULTS AND DISCUSSION

The aerial parts of *A. riparia* afforded, in addition to germacrene D, ten new (**1–10**) and five known (**11–15**) [10, 11] chromenes.

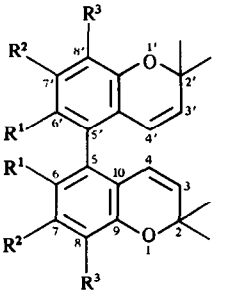
The structure of chromene **1** ($C_{13}H_{16}O_4$, $[M]^+$ 236) directly followed from its molecular formula and ^{1}H NMR spectrum which displayed the typical signals for a 2,2-dimethyl chromene having an unsubstituted C-5 position [10]. The relative positions of the hydroxy and two methoxy groups followed from the observed NOE between the two methoxy groups, between the H-5 and the hydroxy proton as well as from the absence of NOE between H-5 and either of the two methoxy protons. Moreover, NOE was observed between H-4 and the aromatic proton which confirmed a free C-5 position.

The ^{1}H NMR spectrum of chromene **2** ($C_{15}H_{20}O_4$, $[M]^+$ 264) resembled closely that of eupatoriochromene C [12], both showing the presence of identical substituents but different chemical shifts, thereby suggesting that **2** possesed an alternative arrangement of the substituents in the benzene ring. Since the chemical shifts of C-4 and C-5 protons suggested an unsubstituted C-5 position [10] in **2**, the 1-hydroxyethyl and two methoxy groups were tentatively placed at C-6, C-7 and C-8, respectively, leading to the structure **2**. Oxidation of **2** to methylripariochromene A **12** [11], using pyridinium dichromate, confirmed this structure.

Structure **3** ($C_{14}H_{16}O_4$, $[M]^+$ 248) also followed from its ^{1}H NMR spectrum, comparison of which with that of acetovanillochromene **14** [11], clearly showed that one C-2 methyl group of the later was replaced in **3** by a hydroxymethyl group (δ3.86, 2H, *s*). Further confirmation for structure **3** was achieved by NOE experiments where

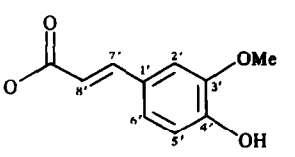


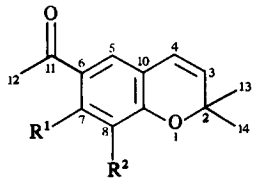
	R^1	R^2	R^3	R^4
1	OH	OMe	OMe	Me
2	CH(OH)Me	OMe	OMe	Me
3	COMe	H	OMe	CH_2OH
4	COMe	H	OMe	CH_2O–ferul.
5	COMe	OH	H	CH_2O–ferul.
6	COMe	OMe	OMe	CH_2OH
7	COMe	OH	OMe	CH_2OH
8	CH(OH)Me	OMe	OMe	CH_2OH
10	COMe	OH	H	CH_2OH
15	$COCH_2OAc$	OH	H	Me
16	$COCH_2OH$	OH	H	Me



9 R^1 = OH, R^2 = R^3 = OMe
9a R^1 = R^2 = OMe, R^3 = OH

ferul. =





riparichromene A **11**: R^1 = OH, R^2 = OMe
methylriparichromene A **12**: R^1 = R^2 = OMe
eupatoriochromene **13**: R^1 = OH, R^2 = H
acetovanillochromene **14**: R^1 = H, R^2 = OMe

clear effects were observed between H-4 and H-5 as well as between the acetyl methyl and both H-5 and H-7.

Chromene **4** ($C_{24}H_{24}O_7$, $[M]^+$ 424) was clearly shown to be a ferulic acid ester of **3** by its ^{1}H NMR spectrum. Accordingly, additional signals for ferulic acid group were observed in the ^{1}H NMR spectrum of **4**. Chromene **5** ($C_{23}H_{22}O_7$, $[M]^+$ 410) was also found to be a ferulic acid ester of a 13-hydroxychromene. Its ^{1}H NMR spectrum agreed well with structure **5** in which the presence of a chelated hydroxy group (due to OH-7) located the ester group at C-13.

The structure of chromene **6** ($C_{15}H_{18}O_5$, $[M]^+$ 278) followed from comparison of its ^{1}H NMR spectrum with that of methylriparichromene A **12** [11]. This structure was further confirmed by the observed NOEs between H-4 and H-5, between H-5 and the acetyl methyl and also between the two methoxy methyls.

The ^{1}H NMR spectrum of chromene **7** ($C_{14}H_{16}O_5$, $[M]^+$ 264), resembled closely that of riparichromene A **11** [10]. The observed NOE between the aromatic singlet and H-4 confirmed the presence of an unsubstituted C-5 position. A chelated hydroxy group was indicated by the ^{1}H NMR and IR spectra of **7**, thereby locating the hydroxy group at C-7 and the methoxy group at C-8, leading to structure **7**.

The structure of chromene **8** ($C_{15}H_{20}O_5$, $[M]^+$ 280), followed directly from comparison of its ^{1}H NMR spectrum with that of **2**. Further confirmation for this structure was achieved by its oxidation with pyridinium dichromate to give chromene **6**.

Compound **9** ($C_{26}H_{30}O_8$, $[M]^+$ 470), isolated as a pale yellow oil, did not contain any aromatic proton in its ^{1}H NMR spectrum. Moreover, the ^{1}H NMR spectrum indicated the presence of two methoxy signals, each containing six protons, doublets for H-3 and H-4, each showing intensity for two protons, and a broad *gem*-dimethyl 12 proton singlet. From these facts together with its M_r the presence of a dimer could be deduced. NOE experiments showed that there were interactions between the two methoxy methyls as well as between one of the methoxy and *gem*-di-methyls which established structure **9**, ruling out the alternative structure **9a**. When **9** was acetylated, the incorporation of two bulky acetyl groups in between the methoxy groups increased the intramolecular interaction resulting in a rotation about the C–C bond between the two benzene rings until a position of minimum interaction was reached. The effect was evident in the ^{1}H NMR spectrum of the acetate where the two *gem*-di-methyl groups were no longer identical and displayed two six proton singlets instead of a broad 12 proton singlet as was present in the parent compound **9**. We have named **9** ageratoriparin.

The roots of *A. riparia* afforded, in addition to dammadienyl acetate, chromenes **6**, **8**–**13**, **15** and **16** [13]. The new compound **10** ($C_{13}H_{14}O_4$, [M] 234) was obtained as a colourless oil. The ^{1}H NMR spectrum of **10** indicated it to be closely similar to eupatoriochromene **13** [10], differing only in having a hydroxymethyl group at C-13 (δ3.64, 2H, *s*) replacing the C-2 methyl group in the latter. Thus, **10** was evidently the alcohol corresponding to ester **5**.

EXPERIMENTAL

400 MHz ^{1}H NMR spectra were recorded in $CDCl_3$ with TMS as int. standard and IR spectra in $CHCl_3$. CI-MS were measured using isobutane as reagent gas. Optical rotations were measured in $CHCl_3$. HPLC was performed using a RP-8 column, flow rate *ca* 2 ml/min, *ca* 100 bar. CC was performed on silica gel (Grade II) and TLC on pre-coated aluminium or glass plates coated with silica gel (GF 254) (layer thickness 1.5 or 0.5 mm). The solvent systems used were A, Et_2O–petrol (1:9); B (1:4); C (2:3); D (1:1); E (3:2); F, C_6H_6–Me_2CO (9:1) and G (8:2).

Isolation of chromenes. Air dried plant material (500 g) (voucher RMK 9316) was chopped and extracted with MeOH–Et_2O–petrol (1:1:1). The resulting extract was first treated with MeOH to remove long chain satd hydrocarbons. The defatted extract was subjected to CC using Et_2O and petrol in different proportions as eluents. The following fractions (200 ml each) were collected: fraction 1 (petrol), fraction 2, Et_2O–petrol (1:9), fraction 3, Et_2O–petrol (3:7), fraction 4, Et_2O–petrol (1:1) and fraction 5 (Et_2O). Fraction 1 afforded 10 mg germacrene D. TLC of fraction 2 (system A, 3 developments) gave 32 mg **13**. Prep. TLC of fraction 3 (system A) followed by TLC (system B) afforded 220 mg **12** and 30 mg **1** (R_f 0.6 in B). Separation of fraction 4 by prep. TLC (system C) followed by TLC (system C, 2 developments) yielded 17 mg **14**, 16 mg **15** and 8 mg **2** (R_f 0.55). Prep. TLC of fraction 5 (system E) gave three major bands. Separations of the least polar band by TLC (system D) yielded 4.5 mg **9** (R_f 0.65). The middle band after two TLC purifications (in systems E and F, respectively) gave 2.6 mg **5** (R_f 0.4 in F). The most polar band after purification by TLC (system G) afforded five crude compounds which were further purified by HPLC using MeOH–H_2O (3:2) to yield 8.6 mg **8** (R_t = 1 min), 6 mg **6** (R_t = 1.5 min), 1 mg **4** (R_t = 3 min) and using MeOH–H_2O (7:3), to afford 2 mg **3** (R_t = 1 min) and 4 mg **7** (R_t = 1.5 min).

The roots of *A. riparia* were worked-up in the same way as aerial parts to give chromatographic fractions 1–5 as mentioned before. Purification of fraction 1 by TLC (system A) gave 16.5 mg dammadienyl acetate. Fraction 2 after TLC (system C) afforded 6 mg **12**, 15.9 mg **13** and 7 mg **11**. Fraction 3 afforded by TLC (system D) sepn, 10 mg **15**. Crystallization of fraction 4 from Et_2O gave 14 mg **16**. TLC of fraction 5 (system F, two developments) yielded 1.5 mg **6**, 2.5 mg **8**, 2.5 mg **9** and 2.5 mg **10** (R_f 0.6).

6-Hydroxyeupatoriochromene B (**1**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 208, 236 sh, 262, 276 sh, 330; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1495, 1475, 1450, 1400, 1385, 1370; MS *m/z* (rel. int.): 236.104 $[M]^+$ (38), 221 $[M-Me]^+$ (100); ^{1}H NMR ($CDCl_3$): δ5.54 (1H, *d*, *J* = 9.5 Hz, H-3), 6.21 (1H, *d*, *J* = 9.5 Hz, H-4), 6.37 (1H, *s*, H-5), 1.45 (6H, *br s*, *gem*-di-Me), 3.9 (3H, *s*, OMe), 3.93 (3H, *s*, OMe), 5.31 (1H, *s*, OH-6).

6-(1-Hydroxyethyl)-eupatoriochromene B (**2**). Colourless gum; $[\alpha]_D^{24}$ = −6.29° ($CHCl_3$; *c* 0.89); UV $\lambda_{max}^{Et_2O}$ nm: 210, 222, 236 sh, 270, 280 sh, 310; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (OH), 1648, 1610, 1470, 1400, 1385, 1365; MS *m/z* (rel. int.): 264.136 $[M]^+$ (10), 249 $[M-Me]^+$ (62), 231 $[249-H_2O]^+$ (100); ^{1}H NMR ($CDCl_3$): δ5.53

(1H, *d*, *J* = 10 Hz, H-3), 6.25 (1H, *d*, *J* = 10 Hz, H-4), 6.72 (1H, *s*, H-5), 5.02 (1H, *q*, *J* = 6.5 Hz, H-11), 1.46 (3H, *d*, *J* = 6.5 Hz, H-12), 1.43 (3H, *br s*, *gem*-di-Me), 3.85 (3H, *s*, OMe), 3.91 (3H, *s*, OMe). Oxidation of **2** (3 mg) with excess of pyridinium-dichromate in CH_2Cl_2 at room temp. (12 hr) followed by TLC (system D) gave 2 mg methylripariochromene A **12**.

13-*Hydroxyacetovanillochromene* (**3**). Colourless oil; $[\alpha]_D^{24} = +12°$ ($CHCl_3$; *c* 0.5); UV $\lambda_{max}^{Et_2O}$ nm: 212, 236, 262 and 312; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1685 (conjugated >C=O), 1610, 1395, 1320; CIMS *m/z* (rel. int.): 249 $[MH]^+$ (100), 217 $[MH - MeOH]^+$ (28); ^1H NMR ($CDCl_3$): δ5.67 (1H, *d*, *J* = 10 Hz, H-3), 6.48 (1H, *d*, *J* = 10 Hz, H-4), 7.44 (1H, *d*, *J* = 2 Hz, H-5), 7.27 (1H, *d*, *J* = 2 Hz, H-7), 2.55 (3H, *s*, H-12), 3.68 (2H, *s*, H-13), 1.45 (3H, *s*, H-14), 3.91 (3H, *s*, OMe).

13-*Feruloyloxyacetovanillochromene* (**4**). Colourless crystals from Et_2O, mp 122.5°; $[\alpha]_D^{24} = +89.6°$ ($CHCl_3$; *c* 0.22); UV $\lambda_{max}^{Et_2O}$ nm: 208, 238, 258, 292 sh, 320; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (OH), 1725 (ester >C=O), 1685 (conjugated >C=O), 1615, 1530, 1480, 1450, 1390 and 1320; MS *m/z* (rel. int.): 224.152 $[M]^+$ (0.5), 217 $[M - CH_2Oferul]^+$ (100); ^1H NMR ($CDCl_3$): δ5.64 (1H, *d*, *J* = 10 Hz, H-3), 6.48 (1H, *d*, *J* = 10 Hz, H-4), 7.46 (1H, *d*, *J* = 2 Hz, H-5), 7.23 (1H, *d*, *J* = 2 Hz, H-7), 2.46 (3H, *s*, H-12), 4.48 (1H, *d*, *J* = 12 Hz, H-13), 4.21 (1H, *d*, *J* = 12 Hz, H-13'), 1.57 (3H, *s*, H-14), 3.91 (3H, *s*, OMe); feruloyl: δ6.16 (1H, *d*, *J* = 16 Hz, H-8'), 7.29 (1H, *d*, *J* = 16 Hz, H-7'), 6.90 (1H, *d*, *J* = 2 Hz, H-2'), 6.87 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.92 (1H, *dd*, *J* = 8.5 and 2 Hz, H-6'), 3.89 (3H, *s*, OMe), 5.85 (1H, *s*, 4'-OH).

13-*Feruloyloxyeupatoriochromene* (**5**). Colourless crystals from Et_2O, mp 147.5°; $[\alpha]_D^{24} = +106.9°$ ($CHCl_3$; *c* 0.13); UV $\lambda_{max}^{Et_2O}$ nm: 206, 246 sh, 258, 280 sh and 326; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3700 (chelated OH), 3520 (OH), 1725 (ester >C=O), 1650 (conjugated >C=O), 1620, 1530, 1390, 1290, 1170; MS *m/z* (rel. int.): 410.136 $[M]^+$ (0.5), 203 $[M - CH_2Oferul.]^+$ (100); ^1H NMR ($CDCl_3$): δ5.5 (1H, *d*, *J* = 10 Hz, H-3), 6.17 (1H, *d*, *J* = 10 Hz, H-4), 7.25 (1H, *s*, H-5), 6.41 (1H, *s*, H-8), 2.41 (3H, *s*, H-12), 4.47 (1H, *d*, *J* = 12 Hz, H-13), 4.12 (1H, *d*, *J* = 12 Hz, H-13'), 1.5 (3H, *s*, H-14), 7.78 (1H, *s*, 7-OH); feruloyl: δ6.17 (1H, *d*, *J* = 16 Hz, H-8'), 7.25 (1H, *d*, *J* = 16 Hz, H-7'), 6.88 (1H, *d*, *J* = 2 Hz, H-2'), 6.87 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.92 (1H, *dd*, *J* = 8.5 and 2 Hz, H-6'), 5.58 (1H, *s*, 4'-OH), 3.89 (3H, *s*, OMe).

13-*Hydroxymethylripariochromene A* (**6**). Colourless gum; $[\alpha]_D^{24} = +14°$ ($CHCl_3$; *c* 0.45); UV $\lambda_{max}^{Et_2O}$ nm: 218, 256, 280; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480 (OH), 1680 (>CO), 1610, 1480, 1430, 1390; CIMS *m/z* (rel. int.): 279 $[MH]^+$ (100), 247 $[MH - MeOH]^+$ (18); ^1H NMR ($CDCl_3$): δ5.58 (1H, *d*, *J* = 10 Hz, H-3), 6.46 (1H, *d*, *J* = 10 Hz, H-4), 7.24 (1H, *s*, H-5), 2.59 (3H, *s*, H-12), 3.69 (2H, *s*, H-13, H-13'), 1.42 (3H, *s*, H-14), 3.88 (3H, *s*, OMe), and 3.97 (3H, *s*, OMe).

13-*Hydroxyripariochromene A* (**7**). Colourless gum; $[\alpha]_D^{24} = +23.6°$ ($CHCl_3$: *c* 0.22); UV $\lambda_{max}^{Et_2O}$ nm: 212, 264, 310, 350; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (chelated OH), 3460 (OH), 1690 (>CO), 1460, 1415, 1320; MS *m/z* (rel. int.): 264.099 $[M]^+$ (4), 233 $[M - CH_2OH]^+$ (100); ^1H NMR ($CDCl_3$): δ5.57 (1H, *d*, *J* = 10 Hz, H-3), 6.44 (1H, *d*, *J* = 10 Hz, H-4), 7.14 (1H, *s*, H-5), 2.55 (3H, *s*, H-12), 3.68 (2H, *s*, H-13, H-13'), 1.43 (3H, *s*, H-14), 3.88 (3H, *s*, OMe).

6-(1-*Hydroxyethyl*)-13-*hydroxyeupatoriochromene B* (**8**). Colourless gum, $[\alpha]_D^{24} = +44.2°$ ($CHCl_3$; *c* 0.84); UV $\lambda_{max}^{Et_2O}$ nm: 210, 275, 284 sh, 310; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460 (OH), 1620, 1470, 1310; MS *m/z* (rel. int.): 280.130 $[M]^+$ (6), 262 $[M - H_2O]^+$ (7), 249 $[M - CH_2OH]^+$ (100), 231 $[249 - H_2O]^+$ (88); ^1H NMR ($CDCl_3$): δ5.54 (1H, *d*, *J* = 10 Hz, H-3), 6.42 (1H, *d*, *J* = 10 Hz, H-4), 6.77 (1H, *s*, H-5), 5.03 (1H, *q*, *J* = 6.5 Hz, H-11), 1.47 (3H, *d*, *J* = 6.5 Hz, H-12), 3.69 (1H, *d*, *J* = 11.5 Hz, H-13), 3.64 (1H, *d*, *J* = 11.5 Hz, H-13'), 1.4 (3H, *s*, H-14), 3.87 (3H, *s*, OMe), 3.93 (3H, *s*, OMe). Oxidation of **8** (4 mg) with pyridinium dichromate in CH_2Cl_2 at room temp. (10 hr) followed by TLC (system E, two developments) afforded 3 mg **6**.

Ageratoriparin (**9**). Light yellow gum; UV $\lambda_{max}^{Et_2O}$ nm: 208, 236 sh, 272, 330; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 1600, 1470, 1435, 1400, 1380, 1150, 1125, 1100; MS *m/z* (rel. int.): 470.194 $[M]^+$ (48), 455 $[M - Me]^+$ (100), 220 $[M/2 - Me]^+$ (18); ^1H NMR ($CDCl_3$): δ5.47 (2H, *d*, *J* = 10 Hz, H-3, H-3'), 5.86 (2H, *d*, *J* = 10 Hz, H-4, H-4'), 3.99 (6H, *br s*, 2 × OMe), 3.95 (6H, *br s*, 2 × OMe), 1.43 (12H, *br s*, 2 × *gem*-di-Me). Acetylation of **9** (4 mg) with Ac_2O–pyridine under reflux (8 hr) followed by usual work-up and purification by TLC (system D) afforded 3 mg acetate; ^1H NMR ($CDCl_3$): δ5.48 (2H, *d*, *J* = 10 Hz, H-3, H-3'), 5.82 (2H, *d*, *J* = 10 Hz, H-4, H-4'), 3.77 (6H, *br s*, 2 × OMe), 3.83 (6H, *br s*, 2 × OMe), 1.41 (6H, *s*, *gem*-di-Me), 1.43 (6H, *s*, *gem*-di-Me), 1.98 (6H, *s*, 2 × OAc).

13-*Hydroxyeupatoriochromene* (**10**). Colourless oil; $[\alpha]_D^{24} = -10°$ ($CHCl_3$; *c* 0.25); UV $\lambda_{max}^{Et_2O}$ nm: 210, 256, 273 sh, 352; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (chelated OH), 3320 (OH), 1650, 1600, 1440, 1396, 1330, 1130, 1090, 1060; MS *m/z* (rel. int.): 234.089 $[M]^+$ (6), 203 $[M - CH_2OH]^+$ (100); ^1H NMR: δ5.52 (1H, *d*, *J* = 10 Hz, H-3), 6.45 (1H, *d*, *J* = 10 Hz, H-4), 7.33 (1H, *s*, H-5), 6.36 (1H, *s*, H-8), 2.54 (3H, *s*, H-12), 3.64 (2H, *s*, H-13, H-13'), 1.41 (3H, *s*, H-14).

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