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# CHROMENES FROM *AGERATINA ARSENII* AND REVISED STRUCTURES OF TWO EPIMERIC CHROMENE DIMERS

NIANBAI FANG,* SANGGONG YU† and TOM J. MABRY

Department of Botany, University of Texas at Austin, Austin, TX 78713-7640, U.S.A.

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**Key Word Index**—*Ageratina arsenii*; Compositae; Eupatorieae; chromenes, *p*-hydroxyacetophenone derivative, revised structures of two epimeric chromene dimers.

**Abstract**—The investigation of the aerial parts of *Ageratina arsenii* afforded eight chromenes and one *p*-hydroxyacetophenone derivative: three of the chromenes are new, namely, agerasanin, 6-[2-(2-hydroxy-4-methyl-5-isopropylphenyl)-ethyl]-7-methoxy-2,2-dimethylchromene and (+)-encecanescin. The structures were elucidated by spectroscopic methods. The structures of two known epimeric chromene dimers, (−)-encecanescin and 9-*epi*-encecanescin, are revised.

## INTRODUCTION

There are numerous reports on the chemistry of the large genus *Ageratina* (Compositae, tribe Eupatorieae) and, on a whole, the chemistry does not appear to be uniform [1, 2]. For example, our group previously showed that *A. tomentella* produced primarily sesquiterpene lactones [3], while *A. saltillensis* yielded diterpenoids [4]. As part of our continuing biochemical systematic investigation of *Ageratina*, we report here from *Ageratina arsenii* (B. L. Robinson), R. M. King, & H. Robinson, eight chromenes, three of which are new, and one known *p*-hydroxyacetophenone derivative. No sesquiterpene lactones or diterpenoids were detected.

## RESULTS AND DISCUSSION

Chromatographic separation of the dichloromethane extracts of *Ageratina arsenii* afforded, in addition to 3,5-bis[isopenten-(2)-yl]-4-hydroxyacetophenone (**9**) [5], five known chromenes (**1**–**5**) as well as three new ones (**6a**, **7** and **8**). The five known chromenes are 6-acetyl-2,2-dimethylchromene (**1**) [6], 6-vinyl-7-methoxy-2,2-dimethylchromene (**2**) [7], 6-(1-methoxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) [7], 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**4**) [8], and vitamin E (**5**). Previously, when (−)-encecanesin, the levorotatory enantiomer of **6a**, and its epimer, 9-*epi*-encecanescin, were reported from *Encelia canescens* they were assigned structures **6b** and **6d** on the basis of $^1$H NMR and mass spectral data [9]. However, additional spectral data reported here, particularly 2D-COSY experiments with aquisition delays, allows the new structural assignment of **6e** and **6c** for (−)-encecanesin and its 9-epimer, respectively; these new assignments are discussed later.

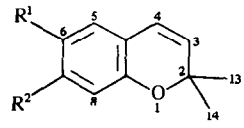
On the basis of $^1$H NMR and $^{13}$C NMR spectral data (Table 1) compounds **1**–**4** were all clearly simple derivatives of chromene, all substituted at position C-6 with a two-carbon moiety. The spectral data also established that **5** was vitamin E.

Except for signals for protons at C-11, 11′ and 12, the $^1$H NMR spectrum of **7** (Table 2) was nearly identical to the combination of the spectra of 6-vinyl-7-methoxy-2,2-dimethylchromene (**2**) and 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**4**). In the $^1$H NMR spectrum of **7**, a characteristic coupling constant of $J_{11,12} = 16.2$ Hz indicated that the H-11 and H-12 were *trans*-oriented (Table 2), which, together with the signal (*ddq*) at $\delta$3.97 for H-11′, indicated that **7** is the C-12/C-11′ condensate of **2** and **4**. The mass spectrum of **7** exhibited a molecular ion at *m/z* 432 (38.8%) in accord with a formula of $C_{28}H_{32}O_4$. Significant fragments at *m/z* 217 (**10**) and 201 appeared to be the same as the dimethyl chromene fragments observed in the spectra of both **2** and **4** [see also ref. 7]. The doublets at $\delta$35 for C-11 and at $\delta$133.4 for C-12 in the $^1$H non-decoupling $^{13}$C NMR spectrum indicated that the two chromenes were linked at C-11′ and C-12. Therefore, the structure of **7** was established and it is given here the name agerasanin.

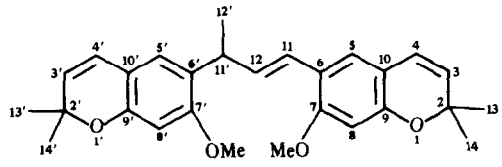
The dimethyl chromene portion ($C_{14}H_{17}O_2$) of **8** was indicated by the mass spectrum which exhibited the same ions as **4** and **7** at *m/z* 217 (**10**) and 201. Since the molecular ion for **8** appeared at *m/z* 366 ($C_{24}H_{30}O_3$), the other part of **8** must be based on $C_{10}H_{13}O$. The $^1$H NMR and $^{13}$C NMR signals for this latter portion clearly indicated that it was a tetrasubstituted benzene moiety substituted with isopropyl, methyl and hydroxyl groups. Moreover, these three groups should be at the C-2′, C-4′ and C-5′ positions since two aromatic signals appeared as two singlets at $\delta$6.52 and 7.12 in the $^1$H NMR spectrum of **8**. The 2D-COSY experiment with aquisition delays (500 MHz) was used to assign the positions of these three groups in the benzene moiety. On the basis of the observed long range coupling of the singlet at $\delta$7.12 with the tetrad at $\delta$4.47 (H-11), the singlet at $\delta$7.12 was

*Permanent address: Hubei College of Chinese Traditional Medicine, Wahan, China.

†Permanent address: Wuhan Institute of Medical Sciences, Wuhan, China.



| | $R^1$ | $R^2$ |
|---|---|---|
| **1** |  | H |
| **2** |  | OMe |
| **3** |  | OMe |
| **4** |  | OMe |



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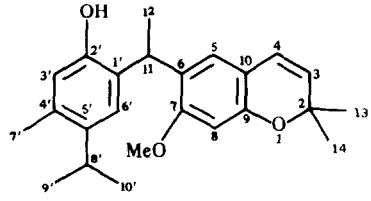

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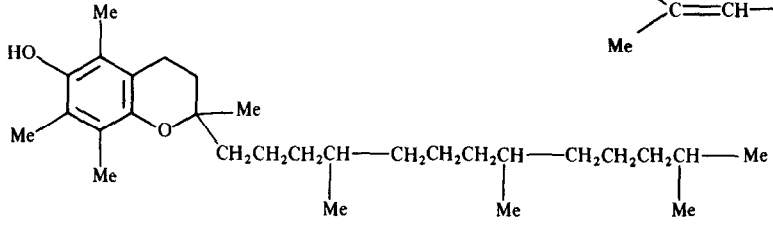

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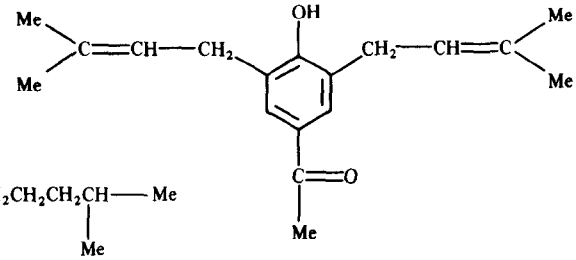

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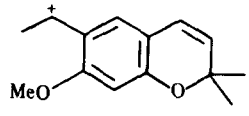

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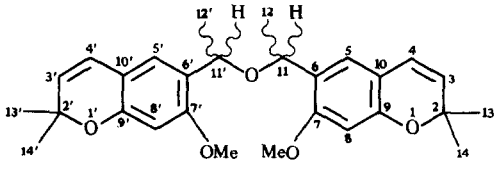
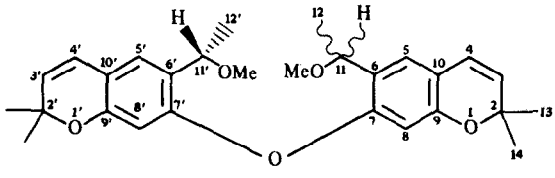


| | | |
|---|---|---|
| **6a** | 11-α-Me | 11'-β-Me |
| **6c** | 11-β-Me | 11'-β-Me |
| **6e** | 11-β-Me | 11'-α-Me |



| | | |
|---|---|---|
| **6b** | 11-β-Me | |
| **6d** | 11-α-Me | |

Table 1. $^{13}C$ NMR spectral data for compounds **1**–**5**, **7** and **9** (360 MHz, $CDCl_3$, $\delta$ scale in ppm)

| C | 1 | 2 | 3 | 4 | 6a | 7 | 9 |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | 130.0 *s* |
| 2 | 77.2 *s* | 76.5 *s* | 75.9 *s* | 76.1 *s* | 76.1 *s* | 76.4 *s* | 128.7 *d* |
| 3 | 121.4 *d* | 121.9 *d* | 121.9 *d* | 121.7 *d* | 122.2 *d* | 122.0 *d* | 134.8 *s* |
| 4 | 126.7 *d* | 124.2 *d* | 123.6 *d* | 123.6 *d* | 124.0 *d* | 124.0 *d* | 157.3 *s* |
| 5 | 129.9 *d* | 128.0 *d* | 127.2 *d* | 127.4 *d* | 127.2 *d* | 127.3 *d* | 134.8 *s* |
| 6 | 130.2 *s* | 119.6 *s* | 123.8 *s* | 125.8 *s* | 125.1 *s* | 119.8 *s* | 128.7 *d* |
| 7 | 130.9 *d* | 157.8 *s* | 157.3 *s* | 157.0 *s* | 157.6 *s* | 157.6 *s* | 197.2 *s* |
| 8 | 115.8 *d* | 99.7 *d* | 99.1 *d* | 99.4 *d* | 99.2 *d* | 99.8 *d* | 26.1 *q* |
| 9 | 157.2 *s* | 154.1 *s* | 152.9 *s* | 153.0 *s* | 152.9 *s* | 153.3 *s* | 29.5 *t* |
| 10 | 120.4 *s* | 114.1 *s* | 113.8 *s* | 113.6 *s* | 113.8 *s* | 114.1 *s* | 121.4 *d* |
| 11 | 196.1 *s* | 131.3 *d* | 72.5 *d* | 65.2 *d* | 68.5 *d* | 125.2 *d* | 127.1 *s* |
| 12 | 25.8 *q* | 111.9 *t* | 22.1 *q* | 22.8 *q* | 23.1 *q* | 133.4 *d* | 17.8 *q* |
| 13 | 28.1 *q* | 28.1 *q* | 27.8 *q* | 27.8 *q* | 28.0 *q* | 28.0 *q* | 25.7 *q* |
| 14 | 28.1 *q* | 28.1 *q* | 27.8 *q* | 27.8 *q* | 28.2 *q* | 28.0 *q* | |
| 1′ | | | | | | | 29.5 *t* |
| 2′ | | | | | 76.1 *s* | 76.1 *s* | 121.4 *d* |
| 3′ | | | | | 122.2 *d* | 122.2 *d* | 127.1 *s* |
| 4′ | | | | | 124.0 *d* | 124.0 *d* | 17.8 *q* |
| 5′ | | | | | 127.2 *d* | 127.9 *d* | 25.7 *q* |
| 6′ | | | | | 125.1 *s* | 126.9 *s* | |
| 7′ | | | | | 157.6 *s* | 157.4 *s* | |
| 8′ | | | | | 99.2 *d* | 99.8 *d* | |
| 9′ | | | | | 152.9 *s* | 125.2 *s* | |
| 10′ | | | | | 113.8 *s* | 113.8 *s* | |
| 11′ | | | | | 68.5 *d* | 35.0 *d* | |
| 12′ | | | | | 23.1 *q* | 20.4 *q* | |
| 13′ | | | | | 28.0 *q* | 28.0 *q* | |
| 14′ | | | | | 28.2 *q* | 28.0 *q* | |
| OMe | | 55.6 *q* | 55.0 *q* | 55.1 *q* | 55.1 *q* | 55.6 *q* | |
| | | | 55.9 *q* | | 55.1 *q* | 55.6 *q* | |

assigned to H-6′. Furthermore, the observed long range coupling between this same singlet at $\delta$7.12 (H-6′) and the heptad at $\delta$3.17 (H-8′) indicated that the isopropyl group is at the C-5′ position while long range coupling between the singlet at $\delta$6.52 (H-3′) and singlet at $\delta$2.07 ($CH_3$) supported a C-4′ methyl group, thereby locating the hydroxyl group at C-2′. These results led to structure **8**. All other spectral data supported the assignment of **8** as 6-[1-(2-hydroxy-4-methyl-5-isopropylphenyl)-ethyl]-7-methoxy-2,2-dimethylchromene.

The $^1H$ NMR spectra of 6-(1-methoxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) and 6-(1-hydroxyethyl)-7-methoxy-2,2-methoxychromene (**4**) showed that the 7-methoxyl group in the benzene moiety of such chromenes occurs at $\delta$3.67–3.70 while the C-11 methoxyl group in the ethyl side chain of these same chromenes appears at $\delta$3.21. That compound **6a** is dimeric could be deduced from the molecular ion at *m/z* 450 for a formula of $C_{28}H_{34}O_5$. Moreover, comparison of $^1H$ and $^{13}C$ NMR, PDFA (one-pulse decouple off during acquisition) of **6a**, with those for 6-(1-methoxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) and 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**4**) suggested that **6a** is the dimer of **4** with an ether linkage between C-11 and C-11′. The only $^{13}C$ NMR signal which distinguished **3**, **4**, and **6a** was for C-11: $\delta$65.2 for **4**, 72.5 for **3** and 68.5 for **6a**. Also, the $^1H$ NMR signal (tetrad) for H-11 ($\delta$5.01 for **4**, 4.66 for **3** and 4.59 for **6a**) and the signal of OMe ($\delta$3.70 for **4**, 3.78 and 3.25 for **3** and 3.67 for **6a**) supported the view that **6a** is the C-11/C-11′ condensate of two monomers of **4**. In the 2D-COSY experiment with acquisition delays, the spectrum of **6a** showed long range coupling between the tetrad at $\delta$4.59 (H-11 and H-11′) and the singlet at 7.10 (H-5) but none between the same tetrad at 4.59 and the singlet at $\delta$3.67 (OMe), which confirmed that the methoxyl group is not at C-11 or C-11′. Furthermore, the long range coupling between the singlet at $\delta$3.67 (OMe) and singlet at $\delta$6.31 (H-8 and H-8′) observed in the 2D-COSY spectrum of (+)-enecanescin confirmed the presence of methoxyl groups at C-7 and C-7′. Thus, the structure of (+)-enecanescin is **6a** and the structure of (−)-enecanescin should be revised from **6b** [9] to **6e**. The same arguments establishes that the structure of 9-*epi*-enecanescin, which was previously isolated from *Encelia canescens* along with (−)-enecanescin [9], should be revised from **6d** [9] to **6c**. The optical results for (+)-enecanescin are: $[\alpha]_D^{25}$, ($CHCl_3$, *c* 0.40):

| 589 | 578 | 546 | 436 nm |
|---|---|---|---|
| +119 | +125 | +143 | +256 |

while Bohlmann and co-workers [9] reported the following results for (−)-enecanescin: $[\alpha]_D^{25}$, ($CHCl_3$, *c* 0.35):

| 589 | 578 | 546 | 436 nm |
|---|---|---|---|
| −113 | −119 | −136 | −246 |

Table 2. $^1$H NMR spectral data for compounds **4**, **7** and **8** (360 MHz, $CDCl_3$, $\delta$ scale in ppm)

| H | 4 | 7 | 8 |
|---|---|---|---|
| 3 | 5.46 *d* ($J$ = 9) | 5.47 *d* ($J$ = 9.8) | 5.40 *d* ($J$ = 9.8) |
| 4 | 6.27 *d* ($J$ = 9) | 6.38 *d* ($J$ = 9.8) | 6.16 *d* ($J$ = 9.8) |
| 5 | 6.94 *s* | 7.08 *s* | 6.48 *s* |
| 8 | 6.36 *s* | 6.36 *s* | 6.34 *s* |
| 11 | 5.01 *q* ($J$ = 6) | 6.65 *dd* ($J$ = 16.2, 1.2) | 4.47 *q* ($J$ = 7.2) |
| 12 | 1.46 *d* ($J$ = 6) | 6.26 *dd* ($J$ = 16.2, 6.8) | 1.47 *d* ($J$ = 7.2) |
| 13 | 1.41 *s* | 1.43 *s* | 1.40 *s* |
| 14 | 1.41 *s* | 1.43 *s* | 1.40 *s* |
| 3′ | | 5.44 *d* ($J$ = 9.6) | 6.52 *s* |
| 4′ | | 6.36 *d* ($J$ = 9.6) | |
| 5′ | | 6.82 *s* | |
| 6′ | | | 7.12 *s* |
| 7′ | | | 2.07 *s* |
| 8′ | | 6.38 *s* | 3.17 *br heptet* ($J$ = 7.0) |
| 9′ | | | 1.26 *d* ($J$ = 7.0) |
| 10′ | | | 1.25 *d* ($J$ = 7.0) |
| 11′ | | 3.97 *dd* ($J$ = 6.8, 1.2, 7) | |
| 12′ | | 1.38 *d* ($J$ = 7) | |
| 13′ | | 1.43 *s* | |
| 14′ | | 1.43 *s* | |
| –OMe | 3.70 *s* | 3.81 *s* | 3.78 *s* |
| | | 3.81 *s* | |

Crystals of (+)-encecanescin suitable for X-ray analysis have not yet been obtained.

## EXPERIMENTAL

*Plant material.* Leaves of *A. arsenii* (1.3 kg) were collected from Morelos, Mexico, between Cuemavaca and Mexica City on March 15, 1986. Voucher specimens (A. McDonald no. 2004) are deposited in the Plant Resources Center at the University of Texas at Austin, Austin, Texas.

*Extraction and isolation of compounds.* The air-dried unground leaves were extracted with $CH_2Cl_2$ for 1 hr and worked-up in the usual manner [10]. The cleaned-up $CH_2Cl_2$ extract (70 g) was chromatographed on a silica gel column packed in hexane–EtOAc (19:1) initiated with the same solvent and gradually increasing to pure EtOAc. Fifteen 500 ml fractions were collected. Further purification for each fraction was achieved by a combination of Sephadex LH-20 chromatography (cyclohexane–$CH_2Cl_2$–MeOH, 7:4:1) and HPLC [semi-prep. silica gel column (10 mm × 25 cm); RI detector; hexane–EtOAc (99:1, 97:3, 19:1 and 9:1) as eluting solvent; flow rate 2.2 ml/min]. Yields: from fraction 12 and 13 compounds **1** (1608 mg) and **9** (38 mg), from fractions 2–7 **2** (65 mg), **3** (476 mg), **5** (744 mg), **6** (85 mg) and **7** (244 mg), and from fractions 8–11 **4** (421 mg) and **8** (7 mg).

*Agerasanin* (**7**). IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 1636, 1618, 1570, 1500, 1468, 1455, 1387, 1365, 1280, 1200; MS *m/z* (rel. int.): 432 $[M]^+$ (20.4), 417 $[M-15]^+$ (100), 227 (10.9), 217 (10.2), 203 (9.6), 201 (40.0), 188 (8.4), 187 (10.6), 185 (9.7), 173 (11.0), 159 (6.7).

6-[2-(2-*Hydroxy*-4-*methyl*-5-*isopropylphenyl*)-*ethyl*]-7-*methoxy*-2,2-*dimethylchromene* (**8**). IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3430 (OH), 1618, 1570, 1570, 1500, 1468, 1458, 1413, 1364, 1272, 1220; MS *m/z* (rel. int.): 366 $[M]^+$ (59.8), 351 $[M-15]^+$ (100), 217 (7.7) 201 (13.1), 189 (13.4), 187 (10.3), 185 (7.0), 175 (9.9), 174 (21.6), 173 (9.4), 169 (8.2), 168 (31.9), 163 (18.2), 115 (12.1), 91 (10.6), 43 (10.6).

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