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COUMARINS FROM *ASTER PRAEALTUS*

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Key Word Index—*Aster praealtus*; Asteraceae; Astereae; coumarins; spinasterol.

Abstract—Chemical analysis of aerial parts and roots of *Aster praealtus* provided, besides the known coumarins umbelliferone, marmin, epoxyaurapten and 6′-hydroxy-β-cycloaurapten, four new 7-*O*-monoterpene ether derivatives of umbelliferone, praealtin A–D. The structures of the new compounds were established by NMR, IR, UV and mass spectral analyses and the molecular structure of praealtin A was determined by single crystal X-ray diffraction. No coumarins were detected in the following taxa: *A. adnatus*, *A. concolor*, *A. dumosus* × *lateriflorus* and *A. lateriflorus*, *A. patens*, *A. spinosus*, *A. subulatus* var. *ligulatus*, and *A. tenuifolius*. *Aster adnatus* provided the Δ^7-sterol, spinasterol, and *A. spinosus* gave the known acetylene lachnophyllum lactone.

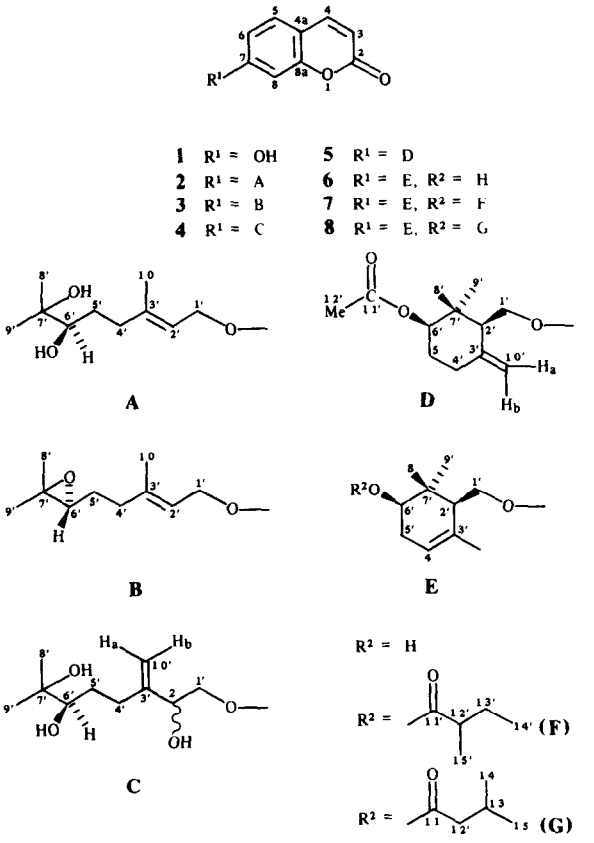
INTRODUCTION

Traditionally, the tribe Astereae of the Asteraceae is divided into six subtribes. *Aster* belongs to the subtribe Asterinae and contains several hundred species [1]. Members of this genus are found worldwide but mainly in the temperate regions of the northern hemisphere. Most species represent herbs and shrubs which are largely perennial and to a lesser extent annual.

Taxonomically, *Aster* is a complex genus due to its variable nature and its tendency towards interspecific hybridization and polyploidy. The systematics of North American species of *Aster* have been recently studied by Jones and co-workers [2–4].

To date, only a relatively small number of *Aster* species have been chemically investigated. Polyacetylenes were isolated from over 50 taxa [5, 6]. A monoterpene was reported from *A. bakeranus* [7] and sesquiterpene hydrocarbons and lactones were found in *A. umbellatus* and *A. exilis* [8]. Diterpenes and diterpene glycosides have been isolated from *A. bakeranus* [7], *A. spathufolius* [9] and *A. alpinus* [10]. Squalene and triterpenes were found in *A. baccharoides* [11], *A. scaber* [12], *A. sikkimensis* [13], *A. tataricus* [14] and *A. bakeranus* [7]. Hydroxycinnamic acids have been isolated from *A. salignus* [15] and a flavone was reported from *A. altaicus* [16], and a benzofuran was found in *A. exilis* [8]. Coumarins were previously detected in 20 *Aster* species [17, 18].

The chemical analysis of *Aster praealtus* Poir from East Baton Rouge Parish, Louisiana resulted in the isolation and structure determination of umbelliferone and seven 7-*O*-monoterpene ether derivatives. The structures of the known coumarins, umbelliferone (**1**) [19], marmin (**2**) [20, 21], epoxyaurapten (**3**) [17] and 6′-hydroxy-β-cycloaurapten (**6**) [17], were established by comparison of their spectral data with those reported in the literature [17]. The structures of the new coumarins **4**, **5**, **7** and **8** were determined by spectral methods (NMR, IR, UV, MS) and the molecular structure of praealtin A (**5**) was established by single crystal X-ray diffraction.



RESULTS AND DISCUSSION

Praealtin D (**4**), $C_{19}H_{24}O_6$, a gum, exhibited peaks in its ^1H NMR spectrum indicating a prenylether derivative

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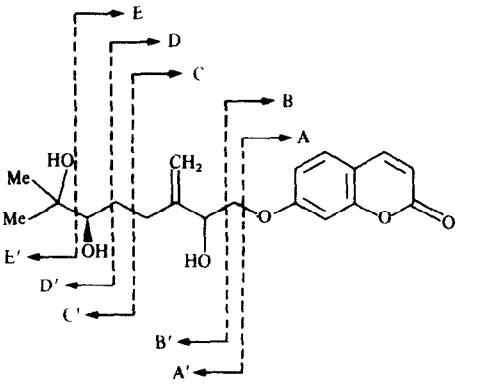
of umbelliferone [17]. A pair of one-proton doublets at δ6.26 ($J_{3,4}$ = 9.5 Hz; H-3) and 7.64 (H-4); H-5 (J = 8 4 Hz) at δ7.38 and H-6 and H-8 at δ6.87 together with UV bands at 219 (sh), 242, 253, 291 (sh) and 322 nm are typical for the coumarin skeleton [22]. The IR spectrum with peaks at 1719, 1709 cm^{-1} (δ-lactone) and 1613, 1557, 1509 (aromatic carbon-carbon stretch) and mass spectral data (Fig. 1) also supported a coumarin structure. The IR spectrum of praealtin (**4**) had a strong absorption band at 3420 cm^{-1}, indicating hydroxyl(s) and the chemical ionization and electron impact mass spectral studies gave a M_r of 348 which is 16 higher than that of marmin (**2**). Praealtin D and marmin (**2**) [20, 21] showed very similar ^{1}H NMR signals for H-4′, H-5′, H-8′ and H-9′. The ^{1}H NMR assignments for **4** (Table 1) were derived from extensive double irradiation experiments and a (^{1}H,^{1}H) COSY-45 with N-type selection experiment [23–25] Instead of the olefinic C-10′ methyl singlet at δ1.78 in marmin (**2**), two broadened one-proton singlets at δ5.09 and 5 27 appeared in the ^{1}H NMR spectrum of praealtin D, suggesting an olefinic methylene group at C-3′. Irradiation of the exocyclic methylene proton at δ5.27 (H-10′a) caused a sharpening of the broadened doublet of a doublet centered at δ4.55 and saturation of the signal at δ4.55 affected the multiplet δ4.06 (H-2′). Similarly, irradiation of the multiplet at δ4.06 collapsed the multiplet at δ4 55 to a broadened singlet. Based on ^{1}H NMR chemical shift considerations and coupling patterns, attachment of a hydroxy group to C-2′ was in agreement with the spectral data and structure **4** is proposed for praealtin D, which represents a new natural product.

The mass spectral fragmentation patterns strongly support this structural arrangement. Figure 1 outlines our assignments of the terpene side chain fragmentation



m/z	Assignment	m/z	Assignment
348 (1 2)*	M^+	162 (100)*	$[AH]^+$
289 (3)	$[E]^+$	155 (32)	$[B'-H_2O]^+$
271 (3)	$[E-H_2O]^+$	103 (2)	$[C']^+$
259 (3)	$[D]^+$	89 (21)	$[D']^+$
246 (8)	$[C+H]^+$	85 (3)	$[C'-H_2O]^+$
245 (1)	$[C]^+$	71 (11)	$[D'-H_2O]^+$
241 (1)	$[D-H_2O]^+$	59 (54)	$[E']^+$
227 (3)	$[C-H_2O]^+$		

* Relative peak intensity

Fig 1 Mass spectral fragmentations (EI) of praealtin D (**4**)

Table 1 ^{1}H NMR data* of praealtin A (**5**), B (**7**), C (**8**) and D (**4**) at 200 MHz in $CDCl_3$ at ambient temperature

H	4	5	7	8
3	6 26 *d* (9 5)	6 25 *d* (9.4)	6 25 *d* (9 4)	6.25 *d* (9.4)
4	7.64 *d* (9.5)	7 64 *d* (9.4)	7 65 *d* (9.4)	7 65 *d* (9.4)
5	7 38 *d* (8.4)	7 37 *d* (8 9)	7 38 *d* (8.5)	7.38 *d* (8.5)
6, H-8	6 87 *m*	6.84 *m*	6.84 *m*	6.84 *m*
1′	4 06 *m*	4 27 *dd* (6 7, 0.7)	4.08 *dd* (10.0, 4.8)	4 08 *dd* (10 0, 4.8)
			4 37 *dd* (10.0, 4 8)	4 36 *dd* (10.0, 4.8)
2′	4 55 *ddd* (7 0, 7 0, 3.5)	2.36 *m*	2.15 *m*	2 15 *m*
4′	2 32 *m*	2 19 *m*	5 39 *br s*	5.38 *br s*
5′	1 65 *m*	1.79 *m*	2.16 *m*	2.16 *m*
			2.05 *m*	2 05 *m*
6′	3 44 *ddd* (10, ~2 5, ~2.5)	4 74 *dd*† (7 8, 7.8)	4 75 *br dd* (5 7)	4.75 *br dd* (5 7)
8′	1 23 *s*	1.08 *s*	1.05 *br s*	1 03 *br s*
9′	1 18 *s*	0.97 *s*		
$10'_a$	5.27 *br s* (3.5)	4 96 *br s* (0 7)	1 79 *br s* (Me)	1.79 *br s* (Me)
$10'_b$	5 09 *br s*	4 72 *br s*		
12′	—	—	2 32 *m*	—‡
13′	—	—	1 57 *m*	—‡
14′	—	—	~0 89 *t*†	~0 93 *d*†
15′	—	—	1 14 *d* (6.8)	
Ac	—	2 11 *s*	—	—

*Chemical shifts are given in ppm relative to Me_4Si Coupling constants (J) or line separations in Hz are given in parentheses

†Multiplicity was obtained from (^{1}H, ^{1}H) J-resolved 2-D spectroscopy

‡Obscured by other signals

and the results of the chemical ionization studies are summarized in the Experimental. The stereochemistry of the hydroxyl group at C-2′ remains open, and the chirality at C-6′ was tentatively assigned *R* based on biogenetic analogy of the chiral centres C-6′ of the known co-occurring coumarins marmin (**2**).

Praealtin A (**5**), $C_{21}H_{24}O_5$, a colourless, crystalline compound (mp 163.3°), exhibited IR signals characteristic of a coumarin: a band at 1734 cm^{-1} was assigned the carbonyl stretch absorption of the unsaturated δ-lactone and peaks at 1615, 1557, 1509 cm^{-1} are due to the aromatic carbon–carbon stretch of the coumarin skeleton. A M_r of 356 was derived from electron impact and chemical ionization mass spectral studies (Experimental). In the ^1H NMR spectrum three one-proton doublets at δ6.25 (H-3), 7.64 (H-4) and 7.37 (H-5), and the two-proton multiplet at 6.84 (H-6 and H-8) were diagnostic of an ether derivative of umbelliferone (Table 1). The basic skeleton of **5** was further corroborated by mass spectral data and diagnostic UV absorptions at 217 (sh), 244, 253, 294 (sh), 322 ($\log \varepsilon = 4.24$). Double irradiation experiments and a (^1H,^1H) COSY-45 with N-type selection experiment were performed to determine proton assignments which are summarized in Table 1. The ^{13}C NMR data for praealtin A are summarized in Table 2. The assignments and multiplicities were deduced from broad-band decoupling, off-resonance decoupling and DEPT experiments and corroborated the ^1H NMR assignments. Based on the above spectral data, structure **5** was proposed for praeltin A, which is a new prenylated coumarin.

Single crystal X-ray diffraction analysis of praealtin A provided the relative configuration of the molecule. Coordinates are given in Table 3 and the molecular structure is illustrated in Fig. 3. The two chiral centres of the monoterpene portion of the molecule are established, with substituents at C-14 and C-18 both equatorial. The six-membered ring of this molecular fragment is in the chair conformation with endocyclic torsion angles ranging in magnitude 49.6–61.4°. The coumarin fused ring system is planar to within 0.044 Å.

Recently, the molecular structure of the structurally related, synthetic (−)-(6′-R)-3′,6′-epoxyaurapten was reported [26]. The conformation of that molecule differs from that of praealtin A primarily in the torsion angle about the C-13/C-14 bond. In praealtin the carbon atom carrying the two methyl groups is *anti* to O12, while in the other compound it is *syn*.

Praealtin B (**7**) *and praealtin C* (**8**), $C_{24}H_{30}O_5$, could not be separated by chromatographic methods. It was a hygroscopic solid, which displayed IR bands characteristic of coumarins: 1734 (C=O stretch of a pyrone ring) and 1615, 1509 cm^{-1} (aromatic C=C stretch). The presence of a C-7 ether derivative of umbelliferone was indicated by absorption bands in the UV spectrum at 219

Table 2 ^{13}C NMR data* of marmin (**2**), epoxyaurapten (**3**), praealtin A (**5**), B (**7**), and C (**8**) in $CDCl_3$ at 50.3 MHz

Assignment	2	3	5	7/8†
2	161.35 *s*	161.16 *s*	161 74 *s*	161.11 *s*
3	112.84 *d*	112.94 *d*	113.54 *d*	113.03 *d*
4	143.55 *d*	143.34 *d*	143.98 *d*	143.31 *d*
4a	112.41 *s*	112.32 *s*	113.03 *s*	112.48 *s*
5	128 72 *d*	128.64 *d*	129 31 *d*	128.72 *d*
6	113 19 *d*	113.09 *d*	113.74 *d*	113.03 *d*
7	162.05 *s*	162.01 *s*	162.68 *s*	161 94 *s*
8	101.53 *d*	101 51 *d*	101.80 *d*	101 26 *d*
8a	155 75 *s*	155 80 *s*	156.46 *s*	155 95 *s*
1′	65 38 *t*	65 28 *t*	67.18 *t*	68 59/68 47 *t*‡
2′	118.78 *d*	119.01 *d*	51 80 *d*	48.91 *d*
3′	142.14 *s*	141.32 *s*	145 11 *s*	133.41 *s*
4′	36.51 *t*	36 17 *t*	30 67 *t*	119.64/119 57 *d*‡
5′	29.43 *t*	27.02 *t*	28.45 *t*	28 80 *t*
6′	77 90 *d*	63 77 *d*	78.25 *d*	75.51/75.32 *d*‡
7′	73.01 *s*	58.29 *s*	38.70 *s*	35 76 *s*
8′	26.41 *q*	24.74 *q*	21.84 *q*	22 66 *q*‡/22.66 *q*†
9′	23.24 *q*	18.67 *q*	19.80 *q*	22 42 *q*‡/22.42 *q*‡
10′	16.71 *q*	16.69 *q*	111.66 *t*	26.10 *q*
11′	—	—	170 86 *s*	172.54/176.10 *s*
12′	—	—	26 77 *q*	41.48 *d*/43.90 *t*
13′	—	—	—	26.80 *t*/25.83 *d*
14′	—	—	—	16.75 *q*/22 34 *q*‡
15′	—	—	—	11 59 *q*/20.15 *q*

*The assignment of the multiplicities are based on off-resonance decoupling and DEPT experiments. Chemical shifts are given in ppm using $CDCl_3$ as an internal standard, Multiplicity for C-4a in **2** and **3** was obtained from DEPT spectra

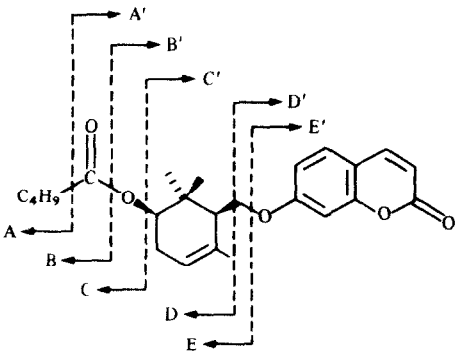
†Chemical shifts and multiplicities for carbon signals of the **7**/**8**-mixture are the same when only one number is shown

‡Assignments for **7** and **8** are interchangeable

Table 3 Coordinates and isotropic thermal parameters for praealtin A

Atom	x	y	z	Biso	Atom	x	y	z	Biso
O-1	0 654 (1)	−0 020 (2)	0.5393 (4)	3.1 (2)	C-14	0.602 (2)	−0.017 (3)	0.2304 (6)	2.8 (3)
C-2	0 703 (2)	−0.017 (3)	0 6031 (7)	3.6 (3)	C-15	0 677 (2)	−0.193 (2)	0 1968 (7)	2.8 (3)
C-3	0 914 (2)	−0 026 (3)	0 6299 (6)	3 4 (3)	C-16	0.684 (2)	−0.166 (3)	0.1271 (7)	4.3 (4)
C-4	1.057 (2)	−0 027 (3)	0 5896 (6)	3 3 (3)	C-17	0.473 (2)	−0.100 (3)	0.0933 (7)	4 1 (4)
C-5	1.144 (2)	−0 028 (3)	0 4762 (6)	3.1 (3)	C-18	0.394 (2)	0.076 (3)	0.1301 (7)	3 4 (4)
C-6	1 080 (2)	−0.032 (3)	0 4123 (6)	2 6 (3)	C-19	0 378 (2)	0.032 (2)	0.2006 (7)	3 4 (4)
C-7	0.876 (2)	−0 028 (3)	0.3913 (6)	3 2 (3)	C-20	0.725 (2)	−0.357 (3)	0.2244 (8)	4.8 (4)
C-8	0 729 (2)	−0 029 (3)	0 4350 (6)	3 3 (3)	C-21	0.299 (3)	0 227 (4)	0.2298 (10)	6.9 (6)
C-9	0 802 (2)	−0.024 (3)	0.4978 (6)	3 0 (3)	C-22	0.234 (2)	−0 138 (3)	0.2107 (7)	4.0 (4)
C-10	1 006 (2)	−0 035 (3)	0 5226 (6)	2.6 (3)	O-23	0 189 (1)	0.112 (2)	0.0992 (5)	4.0 (2)
O-11	0 559 (1)	0 *	0 6363 (5)	4 6 (3)	C-24	0.160 (3)	0 266 (3)	0 0643 (8)	6 0 (5)
O-12	0 828 (1)	−0 032 (2)	0.3268 (4)	3 4 (2)	O-25	0.287 (2)	0.396 (3)	0.0556 (7)	9.3 (4)
C-13	0 613 (2)	−0 031 (3)	0 3019 (6)	3.4 (3)	C-26	−0 071 (3)	0 304 (3)	0.0386 (9)	6 2 (5)

*y Coordinate of O-11 fixed to define the origin.



m/z	Assignment	*m/z*	Assignment
398 (0.7)	[M]$^+$	106 (2)	[**E**'+H−2CO]$^+$
296 (3)	[**C**'−H]$^+$	105 (7)	[**E**'+H−CO−CHO]$^+$
163 (8)	[**E**'+2H]$^+$	85 (43)	[**B**]$^+$
162 (5)	[**E**'+H]$^+$	78 (1)	[**E**'+H−3CO]$^+$
135 (100)	[**E**−**C**−H]$^+$	77 (5)	[**E**'+H−CO−CHO−CO]$^+$
134 (11)	[**E**'+H−CO]$^+$	57 (51)	[**A**]$^+$
121 (21)	[**D**−**C**−H]$^+$		

* % Relative peak intensity

Fig 2 Mass spectral fragmentations (EI) of praealtin B (**7**) and C (**8**)

(sh), 244, 253, 296 (sh) and 322 (log $\varepsilon = 4.25$) and from the mass spectral patterns (fragment E'H in Fig. 2). Furthermore, ^{1}H NMR spectrum showed three doublets at δ6.25 (H-3), 7.65 (H-4) and 7.38 (H-5) and a multiplet at 6.84 (H-6 and H-8) which are all diagnostic for the coumarin nucleus.

The 200 MHz ^{1}H NMR spectrum for the praealtin B/C mixture was very similar to the spectrum for 6'-hydroxy-β-cycloaurapten (**6**). The ^{1}H NMR assignments for **7/8** are summarized in Table 1 and were deduced from extensive spin decoupling experiments and a COSY 45 (^{1}H,^{1}H) experiment with N-type selection. The major difference between the new compounds and coumarin **6** was indicated by the downfield shift of H-6' from δ3.47 in **6** to 4.75 in **7** The more downfield shift of H-6' in **7** suggested that it was attached to a carbon bearing an ester function rather than a hydroxy group. Accordingly, the IR spectrum for **7/8** lacked the absorption for a hydroxy group. A M_r weight of 398 was obtained from electron impact and chemical ionization studies and was in agreement with the molecular formula of the two-component mixture (Fig. 2). The ^{1}H NMR spectrum suggested the presence of a 2-methylbutanoate group in **7**. Saturation of the multiplet at δ2.32 (H-12') collapsed the doublet at δ1 14 ($J = 6.8$ Hz) which was assigned to H-15' Irradiation of the multiplet at δ1.57 (H-13') affected the triplet at 0.89 (H-14'). The ^{1}H NMR data were corroborated by diagnostic mass spectral fragments **A** (*m/z* 57) and **B** (*m/z* 85).

A 400 MHz ^{1}H NMR spectrum in $CDCl_3$ provided clear evidence that two closely related compounds were present even though the gas chromatogram appeared as a

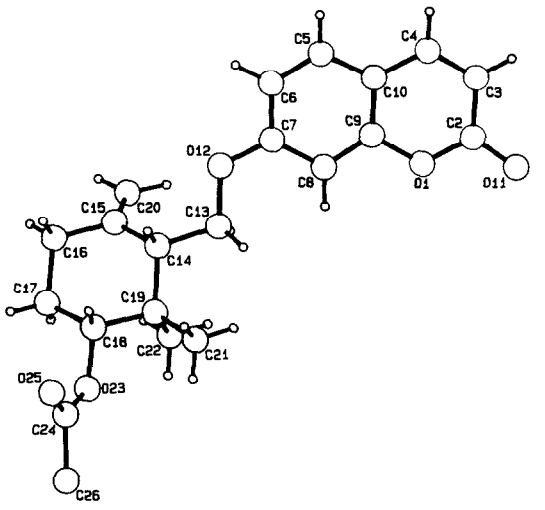


Fig. 3. Molecular structure of praealtin A (**5**)

single peak. Moreover, the difference between the two compounds had to be in the ester functionality. The esters were shown to be isomers by examination of expanded areas of the 400 MHz ^{1}H NMR spectrum and analysis of a 400 MHz (^{1}H,^{1}H) COSY-45 N-type selection spectrum. The ^{1}H NMR signals for the 2-methylbutanoate moiety were assigned as: a three-proton doublet at δ1.04 (H-15′) coupled to the multiplet at 2.30 (H-12′) which in turn was coupled with one of the C-13′ protons at 1.61. The protons attached to C-13′, appearing as multiplets at δ1.61 and 1.42, were coupled with the triplet at 0.84 which was assigned to H-14′. The other ester group had to be an isovalerate group which was indicated by the following characteristic ^{1}H NMR coupling patterns: two methyl groups attached to C-13′ appeared as a doublet of doublets at δ0.89 (J = 6.6 Hz) and 0.88 (J = 6.3 Hz) and were coupled to the multiplet at 2.01 (H-13′). The C-12′ protons appeared as overlapping signals at δ2.12 (J = 7.7 Hz) and 2.12 (J = 6.6 Hz). Therefore, the two compounds differed only in the ester group attached to C-6′, compound **7** being the 2-methylbutanoate (praealtin B) and compound **8** with the isovalerate side chain (praealtin C). These two coumarins are the 2-methylbutanoate and isovalerate derivatives of the known 6′-hydroxy-β-cycloauraptens (**6**) and represent new natural compounds. Final confirmation for the structures came from analysis of the ^{13}C NMR spectrum and the assignments for each compound are summarized in Table 2. The assignments for the ester groups in coumarins **7** and **8** were made by comparison with ^{13}C NMR data reported in the literature [27]. Due to the similarity of the ^{1}H NMR spectral patterns of the terpenoid portion of compounds **7** and **8** with those of the known 6′-hydroxy-β-cycloaurapten (**6**) [17], the same stereochemistry at C-2′ and C-6′ for **7** and **8** was assigned.

Table 4 gives eight further *Aster* species from Louisiana and Texas, which were analysed for the presence of coumarins. The crude terpenoid extracts were obtained by the same method as described for *A. praealtus* (Experimental) and analysed by ^{1}H NMR at 200 MHz for the presence of proton signals diagnostic for coumarins. None of the eight species contained absorptions indicating coumarins and/or terpenoid coumarins. After further chromatographic purifications, *A. adnatus* provided spinasterol and *A. spinosus* gave the acetylene lachnophyllum lactone, which is commonly found in *Aster* species [6].

EXPERIMENTAL

Chemical studies of Aster praealtus. *Aster praealtus* Poir was collected on 3 November, 1983 in East Baton Rouge Parish, Louisiana, U.S.A. (Karla A. Wilzer and Helga D. Fischer, No. 60446; voucher deposited at Louisiana State University, U.S.A.). Dried and ground, aerial parts (400 g) were extracted by soaking with 3 l CH_2Cl_2. After filtration by suction, the solvent was

Table 4. Aerial parts of *Aster* species screened for coumarins

Species	Voucher* number	Collection site	Collection date
A. adnatus Natt.	61607	Tangipahoa Parish, LA	1 November 1984
A. concolor L.	61605	Tangipahoa Parish, LA	1 November 1984
A dumosus × *lateriflorus*	60571	East Baton Rouge Parish, LA	3 November 1983
A lateriflorus (L.) Britt.	61609	East Baton Rouge Parish, LA	25 October 1984
A lateriflorus (L.) Britt.	64999	East Baton Rouge Parish, LA	2 November 1985
A. patens Aiton	61606	Tangipahoa Parish, LA	1 November 1984
A. praealtus Poir	60446	East Baton Rouge Parish, LA	3 November 1983
A praealtus Poir	64364†	East Baton Rouge Parish, LA	31 July 1985
A. spinosus Benth.	1206	Travis County, TX	12 August 1981
A. subulatus Michx. var. *ligulatus* Shinner	61608	East Baton Rouge Parish, LA	2 November 1984
A. subulatus Michx. var. *ligulatus* Shinner	64366	East Baton Rouge Parish, LA	31 July 1985
A. tenuifolius L.	1359	Jackson County, TX	12 September 1981

*Vouchers are deposited at the Louisiana State University Herbarium., except those of *A. spinosus* (S Sundberg No. 1206) and *A. tenuifolius* (S. Sundberg No 1359), which are deposited at the University of Texas at Austin Herbarium.

†This collection of *A. praealtus* was used for chemical analysis of the roots

removed under vacuum to yield 14.2 g of crude extract which was subjected to a previously described procedure [28] to yield 3.6 g of crude syrup The syrup (3.0 g) was pre-adsorbed on silica gel, and fractionated by CC [100 g silica gel, toluene, followed by mixtures of toluene–EtOAc of increasing polarity (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100%)]. 33 100 ml fractions were collected and monitored by TLC. Prep. TLC of fraction 3 (silica gel, hexane–Et_2O, 17.3, × 3) gave 20 5 mg of a mixture of **7** and **8** Fraction 4 (163 mg) contained **3** and **5**, which were separated by prep. TLC (silica gel, petrol–Et_2O, 3 2, × 4) from part of the fraction, CC of the other part on a silanized column (15 g silica gel, petrol–Et_2O mixtures) gave 19 fractions, 25 ml each Fraction 9′ contained **5**, and additional amounts of **3** and **5** were isolated by prep TLC (silica gel, petrol–Et_2O, 3 2, × 4) of fractions 10′–11′

Fraction 5 (222 mg), which contained **3**, **5** and **6**, was chromatographed on 20 g silica gel with hexane–Me_2CO mixtures of increasing polarity taking 17 25 ml fractions Compound **5** was isolated by prep TLC (silica gel, C_6H_6 Me_2CO, 19 1, × 4) from fractions 2′ and 3′ (66 mg) Fractions 4′ and 5′ were combined (86 mg) and provided a mixture of **3**, **5** and 3 5 mg of **6** after prep. TLC (silica gel, petrol EtOAc, 13 7, × 2) A total of 35 mg of pure **3** and 45 mg of **5** were isolated from the various separations

Fractions 8 and 9 were combined (337 mg), and chromatographed over 15 g silica gel, first with hexane–EtOAc mixtures, followed by EtOAc–MeOH mixtures 21 × 25 ml fractions were collected and monitored by TLC Fraction 6′ (145 mg) was further chromatographed by prep TLC (silica gel, petrol–Me_2CO, 7 3, × 3) to yield 14 4 mg of **1**

Fractions 16–19 were combined (270 mg), and chromatographed on a silanized column (20 g silica gel) using petrol–EtOAc mixtures, followed by EtOAc–Me_2CO mixtures 25 × 40 ml fractions were collected and monitored by TLC from which fractions 19′ and 20′ yielded 182 mg **2** Pure **4** (17.2 mg) was obtained from fractions 21′–25′ by prep TLC (silica gel, petrol–Me_2CO, 1·1, × 2)

Praealtin D (**4**) $C_{19}H_{24}O_6$, gum, UV λ_{max}^{MeOH} nm 219 sh, 242, 253, 291 sh, 322; IR ν_{max}^{film} cm^{-1} 3420 (O-H stretch) 3088 (aromatic and olefinic C-H stretch), 2969, 2928, 2876, 2857 sh (aliphatic C-H stretch), 1719, 1709 (unsatd δ-lactone), 1613, 1557, 1509 (aromatic C=C stretch), 1456, 1429 (CH_2 scissoring), 1404, 1352 (C-H bending vibration, CMe_2), 1202 (C-C bending vibration, CMe_2), CIMS (NH_3, probe) *m/z* (rel int) 366 $[M+NH_4]^+$ (9), 349 $[M+H]^+$ (20), 331 $[M+H-H_2O]^+$ (25), 313 $[M+H-2H_2O]^+$ (6), 169 $[A'-H_2O]^+$ (43), 163 $[AH+H]^+$ (100), EIMS (probe) *m/z* (rel. int.) 70 eV 348 $[M]^+$ (1.2).

Praealtin A (**5**) $C_{21}H_{24}O_5$, colourless crystals, mp 163 3° (C_6H_6); UV λ_{max}^{MeOH} nm (log ε) 217 sh, 244, 253, 294 sh, 322 (4.24), IR ν_{max}^{film} cm^{-1} 3083, 3054 (aromatic and olefinic C-H stretch), 2948, 2876, 2857 sh (aliphatic C-H stretch), 1734 (unsatd δ-lactone) 1615, 1557, 1509, (C=C stretch), 1474, 1456, 1429, 1402, 1352, 1374, 1202; CIMS (NH_3, 0.3 T) *m/z* (rel int) 374 $[M+NH_4]^+$ (100), 357 $[M+H]^+$ (3); CIMS (CH_4, 0.3 T) *m/z* (rel. int): 397 $[M+C_3H_5]^+$ (5), 385 $[M+C_2H_5]^+$ (21), 357 $[M+H]^+$ (81), 297 $[M-AcOH+H]^+$ (100), 163 (24), 153 (31), 135 (86)

Praealtin B (**7**) and *Praealtin C* (**8**). $C_{24}H_{30}O_5$, hygroscopic solid, UV λ_{max}^{MeOH} nm (log ε). 219 sh, 244, 253, 296 sh, 322 (4.25); IR ν_{max}^{film} cm^{-1} 3085, 3038 (aromatic and olefinic C-H stretch), 2967, 2936, 2876 (aliphatic C-H stretch), 1734 (carbonyl stretch, unsatd δ-lactone), 1615, 1557, 1509 (C=C stretch), 1462, 1402, 1350, 1370, 1196, CIMS (NH_3, probe) *m/z* (rel int). 416 $[M+NH_4]^+$ (57), 399 $[M+H]^+$ (100), 297 (56), 237 (6 4), 180 (23), 163 (50), 135 (73), EIMS (GC) *m/z* (rel. int) 70 eV 398 $[M]^+$ (0.7)

X-Ray data of praealtin A (**5**) A weakly-scattering crystal of dimensions 0.06 × 0 16 × 0 40 mm was used for data collection on an Enraf–Nonius CAD4 diffractometer equipped with MoKα radiation (λ = 0 71073 Å) and a graphite monochromator. Crystal data are $C_{21}H_{24}O_5$, M_r 356.4, monoclinic space group $P2_1$, a = 6.622(3), b = 6 708(2), c = 21 067(3) Å, β = 96.44(3)°, Z = 2, d_c = 1 273 g/cm³, T = 21°, μ(MoKα) = 0 84 cm^{-1} Data were collected by $\omega-2\theta$ scans of variable rate, varying 0 60–4 0 deg/min One quadrant of data having $1° < \theta < 25°$ was measured, yielding 1771 unique data, of which 987 had $I > 1\sigma(I)$ and were used in the refinement. Data reduction included corrections for background, Lorentz, and polarization effects, absorption effects were insignificant

The structure was solved by direct methods using MULTAN [29], and refined by full matrix least squares based on F, with weights $w = \sigma^{-2}(F_o)$, using the Enraf–Nonius SDP programs [30] Due to the paucity of data, only isotropic refinement was possible. Hydrogen atoms were included as fixed contributions in calculated positions with B = 5 0 Å², except for those of the acetate group, which were not located Convergence was achieved with R = 0.114 using 104 variables, and maximum residual density 0 73 eÅ^{-3} was indicative of anisotropy

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