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TRIHYDROXY-C_{18}-ACIDS AND A LABDANE FROM *RUDBECKIA FULGIDA*

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Key Word Index—*Rudbeckia fulgida*; Compositae; fulgidic acid; 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid; 9,12,13-trihydroxyoctadeca-10(*E*)-enoic acid; labdane; flavone glycosides; C_{14}-polyacetylenes.

Abstract—Extraction of *Rudbeckia fulgida* furnished 13αH-labd-8(17)-en-15-al-19-oic acid, two new C_{18}-acids tentatively formulated as 9 (*S**),12 (*S**),13 (*S**)-trihydroxyoctadeca-10 (*E*),15 (*Z*)-dienoic acid and 9 (*S**),12 (*S**),13 (*S**)-trihydroxyoctadec-10 (*E*)-enoic acid, several known C_{14}-polyacetylenes and several flavone glycosides.

INTRODUCTION

Knowledge of the chemistry of *Rudbeckia* (Heliantheae) is relatively sparse [1–6]. As a result of our discovery of ambrosanolides with antitumour activity in *R. mollis* [6], we have begun a study of other *Rudbeckia* species. In the present article, we report the isolation from *R. fulgida* Ait.* of a new labdane **1a**, the new trihydroxy acids **2a** and **3a**, the known acetylenes **5a–5c**, 2,6-dimethoxybenzoquinone and the flavone glycosides **6a–6e**.

*Our collection of *R. fulgida* represented var. *fulgida* [7].

RESULTS AND DISCUSSION

Detailed analysis of the ^{1}H NMR spectra of the labdane, $C_{20}H_{32}O_3$ (high-resolution mass spectrometry), and its methyl ester led to the formulation of the parent acid as **1a** without commitment as to stereochemistry. The diaxial relationship between the C-10 methyl and the C-4 carboxyl groups was evident from the upfield shift ($\Delta\delta$0.1) of the C-10 methyl signal in going from **1a** to **1b**. To establish the stereochemistry at C-13 and the absolute stereochemistry of the molecule, **1a** was converted to the dimethyl ester **1d**. The ^{1}H NMR spectrum of the latter was identical to that reported for dimethyl oliverate [8]

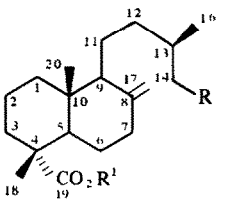
Table 1. ^{1}H NMR spectral data of compounds **2b**, **3b**, **4b** and **4d** (270 MHz, $CDCl_3$)*

H	2b	3b	4b	4d
2†	2.30 *t* (7)	2.30 *t*	2.30 *t*	2.30 *t*
3†	1.59 *m*	1.59 *m*	1.60 *m*	1.62 *m*
4				
7	1.30 *br*	1.28 *br*	1.30 *br*	1.29 *br*
8†	1.59 *m*	1.59 *m*	1.60 *m*	1.62 *m*
9	5.29 *q* (6)	5.21 *q*	5.24 *q*	5.24 *q*
10	5.69 *dd* (15, 6)	5.69 *dd*	5.75 *dd*	5.72 *dd*
11	5.58 *dd* (15, 6)	5.57 *dd*	5.64 *dd*	5.62 *dd*
12	5.37 *t* (6)	5.35 *t*	5.36 *dd* (6, 4)	5.34 *dd*
13	5.03 *q* (6)	5.03 *q*	5.04 *ddd* (8, 6, 4)	5.02 *ddd*
14†	2.30 (obs)	1.50 *q* (*br*) (7)	2.28 (obs)	1.49 *q* (*br*) (7)
15	5.24 (obs)		5.32 (obs)	
16	5.51 *dtt* (10.5, 7, 1.5)	1.28 *br*	5.50 *dtt*	1.26 *br*
17†	2.04 *quintet* (7)		2.02 *quintet*	
18‡	0.96 *t* (7)	0.87 *t* (*br*)	0.97 *t*	0.88 *t* (*br*)
OMe‡	3.68	3.67	3.68	3.68
OAc‡	2.11, 2.07	2.08, 2.06	2.06, 2.06	2.06, 2.06
	2.07	2.05	2.04	2.06

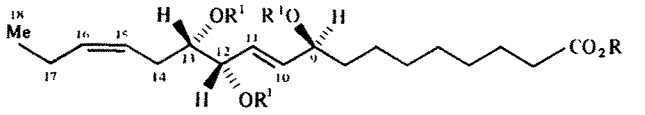
*Coupling constants (in parentheses) are not repeated if identical with those in preceding column.

†Intensity two protons.

‡Intensity three protons.



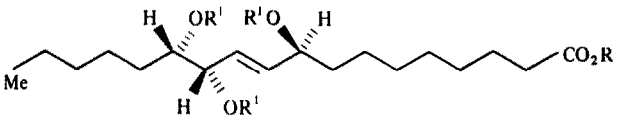
	R	R^1
1a	CHO	H
1b	CHO	Me
1c	CO_2H	Me
1d	CO_2Me	Me



Or mirror image

2a R, R^1 = H

2b R = Me, R^1 = Ac



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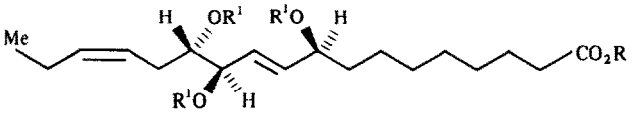
3a R, R^1 = H

3b R = Me, R^1 = Ac

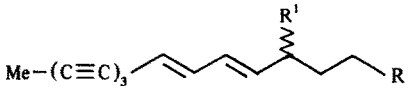


4a R, R^1 = H

4b R = Me, R^1 = Ac

4c 15,16-Dihydro, R, R^1 = H

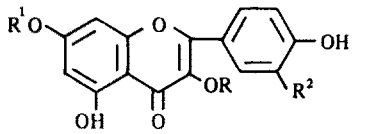
4d 15,16-Dihydro, R = Me, R^1 = Ac



5a R = OAc, R^1 = H

5b R, R^1 = OAc

5c R = OH, R^1 = H

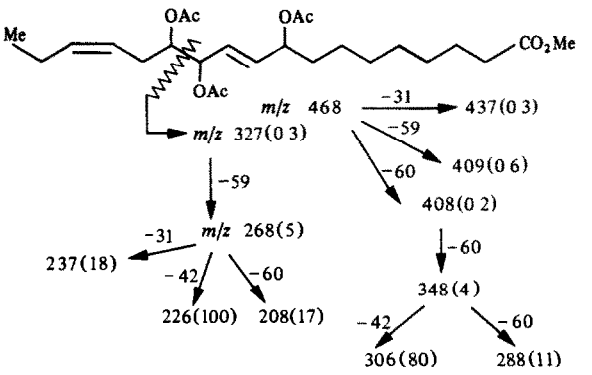


	R	R^1	R^2
6a	rham	H	OH
6b	gluc	H	OH
6c	H	rham	H
6d	gluc	H	H
6e	H	gluc	H

but the rotation had the opposite sign. Hence **1a** was formulated as 13αH-labd-8[17]-en-15-al-19-oic acid.

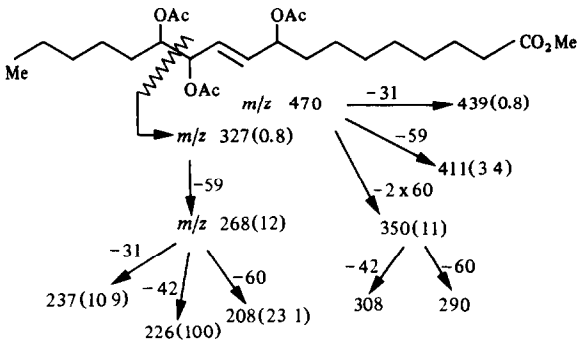
The two acids **2a** and **3a** could be separated with some difficulty only in the form of their derivatives, **2b** and **3b**. Sequential spin decoupling of the ^{1}H NMR spectra of these compounds (Table 1) and mass spectrometry (Schemes 1 and 2) permitted their formulation as a methyl 9,12,13-triacetoxyoctadeca-10(*E*),15(*Z*)-dienoate and a methyl 9,12,13-triacetoxyoctadec-10(*E*)-enoate, respectively. We have named the parent compound (**2a**) fulgidic acid.

The structure and absolute stereochemistry, **4a** (9*S*,12*R*,13*S*), have been deduced recently [9] for malyngic acid from the blue-green alga *Lyngia majuscula*.* Direct comparison of the ^{1}H NMR spectra of **2b** and **3b** with the spectra (see Table 1) of authentic methyl malyngate triacetate (**4b**) and methyl dihydromalyngate triacetate



Scheme 1.

*A malyngic acid sample kindly supplied by Professor R. B. Moore actually was a mixture of malyngic acid and its 15,16-dihydro derivative **4c**. Separation of **4b** and **4d** after methylation and acetylation was achieved as detailed for **2c** and **3c** in the Experimental.



Scheme 2.

(**4d**) clearly demonstrates that the two sets of compounds differ in relative stereochemistry. From the difference in coupling constants involving H-12 and H-13, we infer that fulgidic acid and its 15,16-dihydroderivative are 12,13-*threo*-diols rather than *erythro*-diols like **4a** and **4c**. Moreover, the significant differences in the chemical shifts of H-10, H-11 and H-15 between **2b** and **4b** and H-10 and H-11 between **3b** and **4d** suggest that the spatial relationship between H-9 and H-12 is altered and that fulgidic acid and its dihydro derivative therefore possess the relative stereochemistry 9*S**,12*S**,13*S**. Unfortunately, authentic samples of 9(*S*),12(*R*),13(*S*)- and 9(*S*),12(*S*),13(*S*)-trihydroxystearic acids [9] with one of which we had hoped to correlate **2a** and **3a** were no longer available, so that this proposal must remain tentative.

The results of the present study reinforce the impression that the chemistry of this genus is not very uniform, but further work is clearly needed.

EXPERIMENTAL

Above-ground parts (4.9 kg) of *Rudbeckia fulgida* Ait. var. *fulgida*, collected by Dr. John L. Nelson on 25 September 1982 along U.S. 98, approximately 2 miles east of the Aucilla River, Taylor Co., Florida (Nelson and Wnek 2281 on deposit at the herbarium of the University of South Carolina), were extracted with $CHCl_3$ and worked up in the usual fashion [10]. The crude gum (29 g) was absorbed on 40 g silicic acid (Mallinckrodt 100 mesh) and chromatographed over 600 g of the same absorbent packed in hexane, 500 ml fractions being collected as follows: 1–2 (hexane), 3–8 (hexane–EtOAc, 19:1), 9–14 (hexane–EtOAc, 9:1), 15–18 (hexane–EtOAc, 4:1), 19–22 (hexane–EtOAc, 3:2), 23–26 (hexane–EtOAc, 7:1), 27–30 (hexane–EtOAc, 2:3), 31–34 (hexane–EtOAc, 1:4), 35 and 36 (EtOAc), 37 and 38 (EtOAc–MeOH, 49:1), 39–42 (EtOAc–MeOH, 19:1) and 43–46 (EtOAc–MeOH, 9:1).

Purification of fraction 5 by TLC (C_6H_6–EtOAc, 39:1) afforded 62 mg **5a**, MS *m/z* (rel. int.): 240 $[M]^+$ (99), 198 (4.5), 197 (24), 181 (22), 180 (66), 179 (71), 178 (61.5) and 165 (100); ^{13}C NMR spectrum (67.89 MHz, $CDCl_3$): 4.66 (*q*, C-1), 78.57, 76.62, 75.21, 68.04, 65.00, 59.26 (all *s*, C-2 to C-7), 107.46 (*d*, C-8), 146.27 (*d*, C-9), 130.19 (*d*, C-10), 138.63 (*d*, C-11), 29.24 (*t*, C-12), 27.84 (*t*, C-13), 63.67 (*t*, C-14), 171.03 (*s*) and 20.91 (*q*, OAc). All multiplets were assigned by single frequency decoupling.

Fraction 7 after esterification with CH_2N_2 and purification by TLC (C_6H_6–EtOAc, 39:1, multiple development) yielded methyl stearate (52 mg) and methyl oleate (25 mg). Fractions 11 and 12 contained mainly one substance; purification by TLC (C_6H_6–EtOAc, 19:1) afforded slightly impure **1a** (54 mg); MS *m/z* (rel. int.): 320 $[M]^+$ (3), 274 (8), ^1H NMR (270 MHz, $CDCl_3$): δ9.76 (*t*, *J* = 3 Hz, H-15), 4.84 (*br*) and 4.49 (*br*, H-17a, b), 1.25 (H-18), 0.9 (*d*, *J* = 6.5 Hz, H-16) and 0.60 (H-20). [Calc. for $C_{20}H_{32}O_3$: MW, 320.2351. Found: MW(MS), 320.2351]. Methylation gave spectroscopically pure **1b** as a gum, ^1H NMR (270 MHz, $CDCl_3$): δ9.76 (*t*, *J* = 3 Hz, H-15), 4.87 (*br*) and 4.49 (*br*, H-17a, b), 3.63 (OMe), 1.18 (H-18), 0.97 (*d*, *J* = 6.5 Hz, H-16) and 0.50 (H-20). On standing, **1b** underwent air oxidation to **1c**, which was esterified to **1d** (gum), $[\alpha]_D$ +44° ($CHCl_3$); IR ν^{CHCl_3} cm^{-1}: 1715 *br*; ^1H NMR: δ4.83 (*br*) and 4 48 (*br*, H-17a, b), 3.66, 3.61 (OMe), 1.18 (H-18), 0.94 (*d*, *J* = 6 Hz, H-16) and 0.50 (H-20); MS *m/z* (rel. int.): 364 $[M]^+$, 349 (1), 305 (15.4), 304 (27.7), 289 (4.3). [Calc. for $C_{22}H_{36}O_4$: MW, 364.2614. Found: MW(MS), 364.2643.]

Purification of fraction 13 by TLC (C_6H_6–EtOAc, 9:1) gave 20 mg **5b**; MS *m/z* (rel. int.): 298 $[M]^+$ (6.6), 239 (14.5), 238 (65), 197 (14), 196 (70.5), 195 (87), 179 (55.5), 178 (100), 177 (41.2), 176 (18.7) and 165 (48). TLC of fraction 17 similarly furnished 32 mg **5c**. Fraction 25 on standing in hexane–EtOAc gave 45 mg 2,5-dimethoxybenzoquinone.

Purification of fraction 33 by TLC ($CHCl_3$–MeOH–EtOAc, 8:1:1) gave a fraction which appeared to be homogeneous but whose NMR spectrum showed it to be a mixture of closely related compounds. Esterification (CH_2N_2) and TLC effected no separation. Acetylation in the usual way and separation of the triacetates by TLC (7% $AgNO_3$ on silica gel, C_6H_6–EtOAc, 9:1, 2 developments) gave 15 mg **2b** and 9 mg **3b** as gums ^1H NMR: see Table 1; MS: see Schemes 1 and 2.

Fractions 42 and 43 on standing in EtOAc deposited 0.160 g quercitrin (**6a**). Fraction 44 on purification by TLC ($CHCl_3$–MeOH–EtOAc, 8:1:1) gave a mixture of flavone glycosides. Prep. TLC of the derived acetates ($CHCl_3$–MeOH–EtOAc, 18:1:1) gave two bands. The upper band, on repurification by the above solvent system (2 developments) gave in the upper band a mixture of quercitrin heptaacetate (acetate of **6a**) and astragalin heptaacetate (acetate of **6d**), and in the lower band, a mixture of **6c** heptaacetate and populnin heptaacetate (acetate of **6e**). Repurification of the lower band from the initial TLC purification gave in the upper band a complex mixture of the above compounds; the lower band yielded isoquercetrin octaacetate (acetate of **6b**).

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