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WITHANOLIDES FROM THE FLOWERS OF *DATURA FASTUOSA**

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Key Word Index—*Datura fastuosa* Linn; Solanaceae; Withanolides; withafastuosin F; withametelin; withametelin B; withatatulin B; withatatulin D; withafastuosin E.

Abstract—Withafastuosin F, a new pentahydroxy withanolide and several other known withanolides have been isolated from the flowers of *Datura fastuosa*. The structure of the new compound has been elucidated as 5α,6β,12β,21,27-pentahydroxy-1-oxo-witha-2,24-dienolide from comprehensive spectral analysis.

INTRODUCTION

Our continued interest in *Datura* withanolides in view of their structural novelty and interesting biological activities [1–5] and also due to the observed difference in the oxygenation pattern in withanolides of flowers from those of the leaves [6] prompted us to make an investigation on the flowers of *Datura fastuosa*, the leaves of which yielded several new 21-hydroxy withanolides [7]. Systematic fractionation of the methanolic extract of the fresh flowers of *D. fastuosa* and chromatographic resolution of the different fractions resulted in the isolation of a new withanolide, withafastuosin F (**1**) in addition to several known withanolides previously reported from *D. metel* [8, 9], *D. tatula* [6] and *D. fastuosa* [7].

RESULTS AND DISCUSSION

Withafastuosin F (**1**) $C_{28}H_{40}O_8$ $[MH]^+$ *m/z* 505, mp 246–247° showed spectral features of a typical withanolide; an ultraviolet absorption maximum at 223 nm (ε, 14 300), the diagnostic double triplet at δ 4.46 (J = 13.2, 3.5 Hz) in its ^{1}H NMR spectrum and the carbon resonance signals at δ 205.6 and 167.3, respectively, for the enone and α,β-unsaturated-δ-lactone carbonyl carbons in its ^{13}C NMR spectrum. The 400 MHz ^{1}H NMR spectrum of the compound showed signals for two angular methyls (δ 0.72, 1.24, 3H *s* each), a vinylic methyl (δ 1.99, 3H, *s*) and two primary alcoholic groups—an allyl alcohol in an anisotropic environment (δ 4.24, 4.30, 1H, *d* each, J = 12.3 Hz) and a chiral one attached to a secondary carbon (δ 3.79 1H, *dd*, J = 12.3, 1.7 Hz; 3.88, 1H, *dd*, J = 12.3, 2.9 Hz). The spectrum also showed signals for a steroidal 2-en-1-one system with an unsubtituted methylene at the C-4 position (δ 5.77, 1H, *dd*, J = 10.1, 2.5 Hz; 6.56, 1H, *ddd*, J = 9.8, 5.0, 2.1 Hz). All these signals including a one-proton broad singlet at δ 3.50 were also discernible in the spectrum of withafastuosin E (**2**) [7] and withafastuosin F was considered to be a close relative of compound **2**. Withafastuosin F, however, contains an extra oxygen atom and formed a tetraacetate (**1a**) in contrast to a triacetate (**2a**) given by withafastuosin E. Withafastuosin F was thus considered to be a hydroxy withafastuosin E. The site and position of this hydroxyl group was settled from its ^{1}H NMR spectral analysis. The additional carbinyl hydrogen signal in the spectrum of withafastuosin F (**1**) appeared as a double doublet at δ 3.53 (J = 11, 4.1 Hz) which moved downfield to δ 4.73 in the spectrum of its acetate derivative (**1a**). The only site in the structure of withafastuosin E, where an extra hydroxyl group may be placed so that the resulting carbinyl hydrogen shows such a splitting pattern by coupling with its neighbouring axial and equatorial hydrogens, is C-12. Further, as this hydrogen showed a diaxial coupling, it must be axial and the hydroxyl group must be equatorial and β. The high field position of the 18-methyl carbon (δ 7.5) in contrast to δ 12.5 for the corresponding carbon of withafastuosin E (**2**) indicated a gamma-gauche interaction between the 18-methyl carbon and 12β-hydroxyl group and con-

* Part 32 in the series on withasteroids. For part 31, see reference [5]. For part 29 see reference [2]

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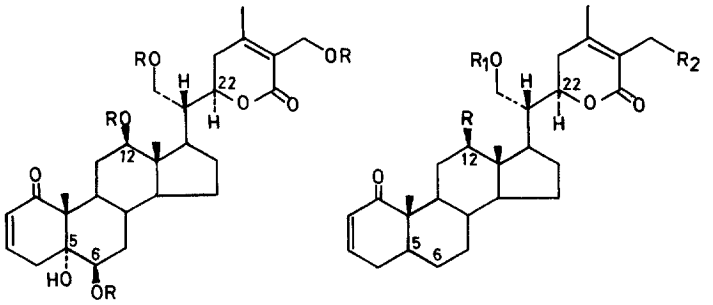

	R
1	H
1a	Ac

		R	R_1	R_2
2	5α-OH, 6β-OH	H	H	OH
2a	5α-OH, 6β-OAc	H	Ac	OAc
3	5β, 6β-epoxy	OH	H	H

Table 1. ^{13}C NMR chemical shift assignments of compounds **1**, **1a**, **2a** and **3**

C	**1**	**3** [6]	**1a**	**2a** [7]
1	205.6	—	202.8	203.7
2	128.0	—	128.8	128.9
3	142.3	—	140.4	140.3
4	35.3	—	35.3	35.4
5	78.4	—	76.1	76.3
6	73.8	—	75.1	75.3
7	32.3	—	32.9	32.5
8	28.6	28.8	29.2	30.7
9	46.5	43.3	40.7	41.1
10	51.6	47.8	51.6	51.8
11	32.8	32.8	29.9	23.2
12	78.3	78.1	79.9	38.7
13	48.1	48.0	47.9	42.8
14	53.9	54.3	53.2	55.2
15	23.7	23.8	23.4	24.1
16	28.6	28.7	26.0	27.3
17	46.6	46.2	46.6	46.5
18	7.5	7.3	8.7	12.5
19	15.3	—	14.9	15.2
20	39.4	—	39.1	42.6
21	56.4	—	57.9	58.0
22	—	—	76.9	76.9
23	28.9	—	29.7	30.1
24	155.5	—	157.2	157.6
25	124.9	—	121.9	121.8
26	167.3	—	164.8	164.9
27	58.4	—	62.8	61.6
28	19.8	—	20.6	20.6
-OAc		—	171.2, 170.9 170.2, 169.9, 21.7, 21.4, 21.0, 20.9	170.9, 170.7, 170.0 21.4, 21.1, 20.9

firmed its β-orientation. Withafastuosin F was thus proved to 12β-hydroxy-withafastuosin E (**1**). This structure was also consistent with its ^{13}C NMR data; while the resonance signals of the AB ring and side-chain carbons were found to be in perfect agreement with those of compound **2**, the C and D ring carbons resonated almost at the same field as those of withatatulin B (**3**), a 12,21-dihydroxy withanolide [6].

Withafastuosin F (**1**) is a new addition to the group of withanolides having hydroxyl groups both at C-12 and C-21 positions, the first members of which were isolated from the flowers of *Datura tatula* [6].

EXPERIMENTAL

General procedures have been described previously [10].

Extraction and isolation of the compounds. Fresh flowers of *D. fastuosa* (6 kg) were crushed and then kept soaked in MeOH in a percolator. After 1 week, the extract was drained, concentrated under red. pres. and the process was repeated twice. The combined extract (*ca.* 600 ml) was diluted with an equal volume of H_2O and extracted exhaustively with EtOAc. The EtOAc-soluble fraction (35 g) was chromatographed over silica gel and the column was eluted with solvents of increasing polarity. The frs eluted with C_6H_6-EtOAc (3:1) yielded withametelin (**1**, 10 mg) [8] and withametelin B (**2**, 20 mg) [9] and those eluted with C_6H_6-EtOAc (1:3) yielded withatatulin B (**3**, 1 g), withatatulin D (7 mg) [6] and withafastuosin E (**2**, 0.7 g) [7], on rechromatography over silical gel. The frs eluted with EtOAc-MeOH (19:1) were rechromatographed over silica gel and eluted initially with EtOAc and then with EtOAc-MeOH mixtures of increasing polarity. Withafastuosin F (**1**, 0.3 g) was isolated from EtOAc-MeOH (24:1) eluates.

Withafastuosin F (**1**). Crystallization from an EtOAc-MeOH mixture yielded white needles, mp 246–247°; UV λ_{max} nm (log ε): 223 (4.16); ^{13}C NMR (Table 1); FABMS *m/z*: 505 $[MH]^+$, 487 $[MH\text{-}H_2O]^+$, 469 $[MH\text{-}2H_2O]^+$, 451 $[MH\text{-}3H_2O]^+$.

Withafastuosin F tetraacetate (**1a**). Withafastuosin F (0.1 g) was acetylated in the usual way with Ac_2O-pyridine at reflux temp. and purified by CC to yield a crystalline compound (**1a**, 75 mg), mp 152–153°; ^1H NMR: δ 6.55 (1H, *ddd*, J = 10.1, 5, 2.1 Hz, H-3), 5.87 (1H, *dd*, J = 10.1, 2.5 Hz, H-2), 4.85 (2H, AB*q*, J = 11.6 Hz, H_2-27), 4.79 (1H, *t*, J = 2.4 Hz, H-6), 4.73 (1H, *dd*, J = 10.9, 4.4 Hz, H-12), 4.46 (1H, *dt*, J = 13.2, 3.3 Hz, H-22), 4.38 (1H, *dd*, J = 11.7, 3.3 Hz, H_a-21), 4.07 (1H, *dd*, J = 11.7, 5.2 Hz, H_b-21), 2.85 (1H, *dt*, J = 19.8, 2.5 Hz, H_a-4), 2.11, 2.06, 2.04, 2.01 (3H, *s* each, 4-OAc), 1.98 (3H, *s*, H_3-28), 1.24 (3H, *s*, H_3-19), 0.87 (3H, *s*, H_3-18), FABMS *m/z*: 695 $[M.Na]^+$, 673 $[MH]^+$, 553 [MH-2AcOH], 535 [MH-2 AcOH-H_2O]$^+$.

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