Pergamon

0031-9422(95)00699-O

Phytochemistry, Vol. 41, No. 3, pp. 981–983, 1996

0031-9422/96 $15.00 + 0.00

WITHANOLIDES FROM *DATURA TATULA**

M. MANICKAM, S. B. AWASTHI, A. SINHA-BAGCHI,† S. C. SINHA† and A. B. RAY‡

Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India; †Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La-Jolla, CA 92037, U.S.A.

(*Received* 19 *June* 1995)

Key Words Index—*Datura tatula*; Solanaceae; C_{21}-oxygenated withanolides; withatatulin; withametelin; withametelin F; withametelin H.

Abstract—Withatatulin, a new 21-hydroxy withanolide and several other known withanolides have been isolated from the leaves of *Datura tatula*. The structure of the new compound has been elucidated from comprehensive spectral analysis and by establishing its chemical relationship with a withanolide of known structure. Physalindicanol A, a C_{28}-sterol has also been isolated from this source.

INTRODUCTION

Withanolides occupy an important position in the growing groups of steroidal lactones, known as withasteroids [1]. *Datura* is one of the few selected genera of the family Solanaceae that are known to elaborate withanolides. A common feature of the *Datura* withanolides earlier reported from *D. quercifolia*, *D. ferox*, *D. stramonium* and several *Datura* hybrids [1–3] is the presence of a 5α-hydroxy-6α,7α-epoxy-2-en-1-one moiety. Occasionally, these compounds are found to be oxygenated at C-12 and bear an epoxylactone ring in the side chain, and in this respect *Datura* withanolides may be regarded as close relatives of *Nicandra* withanolides that are built on intact ergostane framework [1, 4, 5]. A departure from this typical substitution pattern was first witnessed in withametelin (**1**), the major withanolide of *Datura metel* [6, 7] and the first withanolide with an oxygen substituent at C-21. An oxygen substituent at C-21 was later found to be rather a common feature of the withanolides of *D. metel* and *D. fastuosa* [1, 8]. We report here the isolation of a new withanolide, withatatulin (**2**) from the leaves of *D. tatula* [9] and its structure determination. The isolation of several known compounds withametelin (**1**), withametelin F [10], withametelin H [11] and physalindicanol A [12] from this source is also reported.

RESULTS AND DISCUSSION

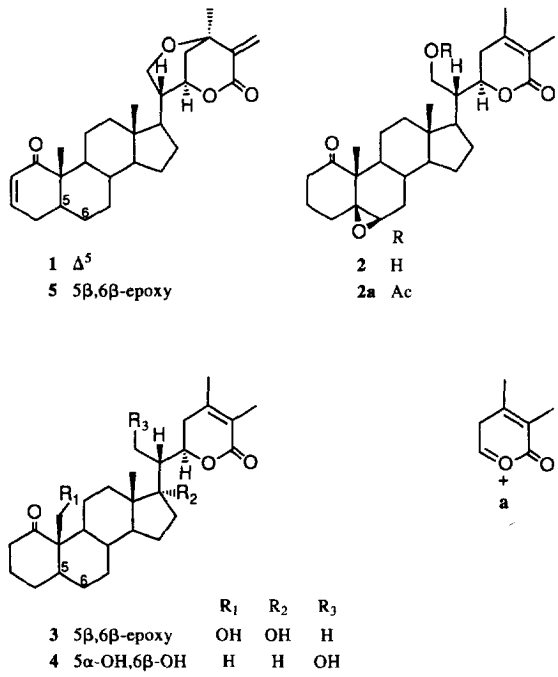
Withatatulin (**2**) $C_{28}H_{40}O_5$ $[M]^+$ *m/z* 456, mp 288–290° displayed diagnostic spectral features of a typical withanolide; a base peak at *m/z* 125 for ion **a** in its electron-impact mass spectrum and a one-proton double triplet at δ4.43 (J = 13.4,3.4 Hz) in its ^{1}H NMR spectrum [1]. The most significant feature of the ^{1}H NMR spectrum of withatatulin is the conspicuous absence of olefinic hydrogen signals of the 2-en-1-one double bond of withanolides and the appearance of only four methyl signals as singlets, two for angular methyls and the remaining two for vinylic methyl groups. The UV, IR and ^{13}C NMR spectra of withatatulin indicated the presence of an α,β-unsaturated-δ-lactone (ν_{max} 1674 cm^{-1}, δ_C 166.8) and a non-conjugated keto-carbonyl function (ν_{max} 1712 cm^{-1}, δ_C 213.2). In accordance with the IR spectral band at 3530 cm^{-1} which indicated the presence of hydroxyl group in the molecule, withatatulin yielded a monoacetate (**2a**) on acetylation with Ac_2O–pyridine, and a comparison of the ^{1}H NMR spectrum of this acetate derivative with that of the parent molecule revealed a downfield shift of two one-proton signals; a broad doublet at δ4.03 and a double doublet at δ3.76 moved down respectively to δ4.29 (1H, *dd*, J = 12.0, 2.4 Hz) and δ4.16 (1H, *dd*, J = 12.0 and 4.8 Hz). That these two hydrogen signals represent AB protons of an ABX system was apparent from their splitting pattern. This observation taken, in conjuction with the oxymethylene carbon signal at δ59.7 in the ^{13}C NMR spectrum suggested the presence of a >CH–CH_2OH grouping in withatatulin which in turn explained that the missing C-21 methyl group of the withanolide is replaced by a hydroxymethyl. The carbinyl hydrogen signal appearing as a broad singlet at δ3.11 in the ^{1}H NMR spectrum of the compound remained virtually unaffected on acetylation and this was assigned to H-6 of a steroidal 5β,6β-epoxide from a comparison of its ^{1}H NMR parameters with the reported values of H-6 of withanolides having similar system [8, 10, 11]. The structure of withatatulin was thus deduced as **2**, which received

*Part 27 in the series on withasteroids. For part 26, see reference [8].

‡Author to whom correspondence should be addressed.

Table 1. ^{13}C chemical shift assignments of compounds **2**, **3** and **4**

C	2	3 [13]	4 [14]
1	213.2	210.7	
2	32.3	31.4	
3	31.8	31.1	
4	35.2	35.6	
5	64.3	64.1	
6	60.5	59.3	
7	20.5	21.5	
8	29.2	30.6	
9	42.9	42.4	
10	52.2	55.9	
11	22.0		23.1
12	38.6		37.2
13	42.3		39.4
14	55.8		55.5
15	24.1		24.1
16	27.2		29.6
17	46.2		47.0
18	11.9		12.4
19	13.2		19.9
20	45.1		43.0
21	59.7		58.8
22	77.9		77.9
23	30.3		30.6
24	150.2		150.6
25	121.6		121.1
26	166.8		166.3
27	12.4		12.9
28	18.3		20.9



support from its ^{13}C NMR spectral analysis. The resonance signals of the AB ring carbons of **2** matched well with those of jaborosalactone O (**3**) [13] and the resonance signals of the C and D rings and side chain of **2** were in fair agreement with the corresponding carbons of withametelin C (**4**) [14] (Table 1).

Our previous experience with catalytic hydrogenation of withametelin (**1**) which cleaved the allyl ether bridge of the bicyclic side-chain with concomitant reduction of the enone double bond [6] prompted us to provide chemical evidence in support of the structure of withatatulin. Hydrogenation of withametelin F (**5**) in the presence of Pd-C catalyst gave a mixture that, on chromatographic resolution, yielded withatatulin as the major product.

D. tatula is the third solanaceous plant known to elaborate C-21-oxygenated withanolides.

EXPERIMENTAL

Mps were determined in open capillary and are uncorr. UV spectra were recorded in MeOH on a JASCO UV-Vis 7800 instrument and IR spectra on a FT IR-5300 spectrophotometer. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken with a Brucker AMX 400 spectrometer using $CDCl_3$ solutions. CC and TLC were carried out respectively, over silica gel (60–120 mesh) and silica gel G (Qualigens Fine Chemicals). C_6H_6–EtOAc (1:1) was used for developing TLC plates. The known compounds were identified by direct comparison (mmp, Co-TLC, MS, ^1H NMR) with authentic samples.

Plant material. D. tatula L. which grows in the sub-Himalayan tracts of India is often cultivated in gardens because of its large purple flowers. The plants used in the present investigation were raised from the seeds collected from the garden of Chunar Fort, Mirzapur, Uttar Pradesh, India. The plant was identified by Prof. G. N. Choudhury, Department of Botany, Banaras Hindu University. A voucher specimen of the plant is kept in the department.

Extraction and isolation of the compounds. Powdered leaves of *D. tatula* (2 kg) were extracted with MeOH in a Soxhlet apparatus. The extract was concd under red. pres. to a thick syrup (250 g), diluted with H_2O (200 ml) and extracted successively with petrol (60–80°) and $CHCl_3$. The petrol fr. was freed from solvent and chromatographed over silica gel and the column was eluted with solvents of increasing polarity. The frs eluted with C_6H_6–EtOAc (1:1) provided a green gummy residue (7 g) which was rechromatographed over a bed of silica gel. Elution of the column with C_6H_6–EtOAc (3:1) yielded withametelin (**1**, 10 mg) from early frs, withametelin F (**5**, 20 mg) from middle frs and physalindicanol A (30 mg) from late frs. Withatatulin (**2**, 150 mg) was isolated from C_6H_6–EtOAc (1:1) eluates.

The $CHCl_3$ fr. was concd and chromatographed over silica gel and the column was eluted initially with C_6H_6 and then with C_6H_6–EtOAc mixtures of increasing polarity. Withametelin H (70 mg) was obtained from C_6H_6–EtOAc (1:1) eluate.

Withatatulin (**2**). Crystallization from EtOAc yielded microcrystalline powder, mp 288–290°; UV λ_{max} nm (log ε): 228 (4.01); IR ν_{max} cm^{-1}: 3530, 1712, 1674; ^1H NMR: δ4.43 (1H, *dt*, J = 13.4, 3.4 Hz, H-22), 4.03 (1H, *br d*, J = 11.5 Hz, H_a-21), 3.79 (1H, *dd*, J = 11.5, 5.1 Hz, H_b-21), 3.13 (1H, *br s*, H-6), 1.93, 1.87, 1.17, 0.70 (3H, *s* each, Me-28, 27, 19, 18); ^{13}C NMR (Table 1); FABMS *m/z*: 479 $[M + Na]^+$; EIMS *m/z*: 456 (base peak), 125 (72%).

Withatatulin monoacetate (**2a**). Withatatulin (0.1 g) was acetylated in the usual way with Ac_2O–pyridine at ambient temp. and purified by CC to yield an amorphous powder (70 mg), mp 203–205°; ^1H NMR: δ4.39 (1H, *dt*, J = 13.5, 3.5 Hz, H-22), 4.29 (1H, *dd*, J = 12.1, 2.4 Hz, H_a-21), 4.16 (1H, *dd*, J = 12.0, 4.5 Hz, H_b), 3.11 (1H, *br s*, H-6), 2.02 (3H, *s*, -OAc), 1.92, 1.85, 1.14, 0.68 (3H, *s* each, Me-28, 27, 19, 18); CIMS *m/z*: 499 $[MH]^+$.

Conversion of withametelin F (**5**) *to withatatulin* (**2**). A soln of withametelin F (30 mg) in MeOH (10 ml) was stirred with 5% Pd-C (20 mg) in an atmosphere of hydrogen at ambient temp. till the consumption of hydrogen ceased. The reaction mixture was freed from catalyst and organic solvent and the residue was chromatographed over a short bed of silica gel. Elution of the column with C_6H_6–EtOAc (1:1) yielded withatatulin (7 mg), indistinguishable from the natural product by direct comparison (mmp, Co-TLC, MS, ^1H NMR).

Acknowledgements—Financial assistance from CSIR, New Delhi is gratefully acknowledged. Thanks are also due to Dr M. Gupta for collection of seeds of the plants.

REFERENCES

1. Ray, A. B. and Gupta, M. (1994) *Prog. Chem. Org. Nat.* Vol. 63, pp. 1–106. Springer-Verlag. Vienna.
2. Dhar, K. L. and Raina, M. L. (1973) *Phytochemistry* **12**, 426.
3. Evans, W. C., Grout, R. J. and Menash, M. I. K. (1984) *Phytochemistry* **23**, 1717.
4. Bagchi, A., Neogi, P., Sahai, M., Ray. A. B., Oshima, Y. and Hikino, H. (1984) *Phytochemistry* **23**, 853.
5. Begley, M. J., Crombie, L., Ham, P. J. and Whiting, D. A. (1976) *J. Chem. Soc. Perkin Trans I*, 296.
6. Sinha, S. C., Kundu, S., Maurya, R., Ray, A. B., Oshima, Y., Bagchi, A. and Hikino, H. (1989) *Tetrahedron* **45**, 2165.
7. Oshima, Y., Bagchi, A., Hikino, H., Sinha, S. C., Sahai, M. and Ray, A. B., (1987) *Tetrahedron Letters* **28**, 2025.
8. Manickam, M., Awasthi, S. B., Oshima, Y., Hisamichi, K., Takeshita, M., Sahai, M. and Ray, A. B. (1994) *J. Chem. Res.* (S), 306.
9. Duthie, J. H. F. (1960) in *Flora of Upper Gangetic Plain*, Vol. 2, p. 9. Botanical Survey of India, Calcutta.
10. Jahromi, M. A. F., Manickam, M., Gupta, M., Oshima, Y., Hatakeyama, S. and Ray, A. B. (1993) *J. Chem. Res.* (S) 234.
11. Manickam, M., Kumar, S., Sinha-Bagchi, A., Sinha, S. C. and Ray, A. B. (1994) *J. Ind. Chem. Soc.* **71**, 393.
12. Sinha, S. C., Ali, A., Bagchi, A., Sahali, M. and Ray, A. B. (1987) *Planta Medica* 55.
13. Monteagudo, E. S., Burton, G., Gros, E. G., Gonzalez, C. M. and Oberti, J. C. (1989) *Phytochemistry* **28**, 2514.
14. Gupta, M., Manickam, M., Sinha, S. C., Sinha-Bagchi, A. and Ray, A. B. (1992) *Phytochemistry* **31**, 2423.