WITHANOLIDES OF Datura stramonium

II. WITHASTRAMONOLIDE

R. N. Tursunova, V. A. Maslennikova,
and N. K. Abubakirov

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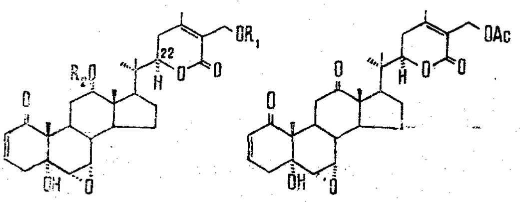
We have previously [1] reported the isolation from the leaves of Datura stramonium L. of daturalactone and another withanolide with a molecular weight two units less than that of daturalactone which we prvisionally called stramonolide [2]. We have investigated a plant collected in the flowering period in the botanical garden of the Academy of Sciences of the Uzbek SSR. It differed from the usual datura by light violet bands within the flower and leaves nonuniformly colored reddish violet. In its other morphological characteristics, the colored form – D. stramonium f. violacea – did not differ in any way from the basic form [3]. At the same time, as is not surprising, the basic form free from anthocyan color contained no withanolides. This is one more example of the wide polymorphism of those plants of the family Solanaceae that are capable of producing withanolides. The multinationality of the forms was shown most clearly in an investigation of the withanolides of the plant Withania somnifera Dun. [4, 5].

In the present paper we discuss the determination of the structure of a compound more polar than daturalactone which has acquired the name of withastramonolide (I). The empirical formula of the new withanolide, $C_{20}H_{38}O_7$, was established with the aid of mass spectrometry (M^+ 486). Analysis of the mass spectrum also confirms the presence of the basic steroid skeleton and of an unsaturated lactone ring in the side chain. The presence of ions with m/e 141 ($C_7H_9O_3$) and 345 ($C_{21}H_{29}O_4$) shows the cleavage of the bonds at C-20 and C-22 that is characteristic for withanolids. Besides those already mentioned, the spectrum of withastramonolide contains fragments with m/e 468, 450, and 432, showing the presence of three hydroxy groups, while the formation of ions with m/e 141 and 123 (141 – H_2O, 100%) shows that one of the hydroxy groups is present in the lactone ring. This conclusion is in complete harmony with the features of the NMR spectrum of the withanolide itself and its monoacetate (II). A two-proton singlet from two equivalent methylene protons at C-27 of withastramonolide (I) appears at 4.42 ppm, as in the spectra of the jaborosalactones [6] and of withaferin A [7]. In the acetate of (II), the signal is shifted downfield, to 4.89 ppm. Hence, it may be concluded that the hydroxy group in the lactone ring is present at C-27. The almost instantaneous occurrence of the acetylation reaction is new evidence in favor of the assumption that the hydroxy group is primary.

The strong absorption of the withanolide in the UV spectrum at 225 nm (log ε 4.17), bands at 1690 and 1675 cm^{-1} in the IR spectrum, weak-field signals of the C-2 and C-3 vinyl protons (5.82 and 6.6 ppm), and also the signals from the C-6 and C-7 protons (doublets at 3.05 and 3.35 ppm) in the NMR spectrum agree well with the analogous indices of known 5α-hydroxy-1-oxo-6α-7α-epoxy-Δ^2-steroids [8, 9]. The α orientation of the hydroxy group at C-5 finds confirmation in the circular dichroism spectrum of withastramonolide (I) with a well-defined negative sign of the Cotton effect in the 337 nm region (Δε –1.9).



I, $R_1 = R_2 = H$
II. $R_1 = Ac$; $R_2 = H$
III. $R_1 = R_2 = Ac$

IV

The NMR spectra of compounds (I) and (II) contain at 4.0 ppm a one-proton signal with $W_{\frac{1}{2}} = 5$ Hz, due to an equatorial hydrogen atom geminal to a hydroxy group. The secondary nature of the latter is confirmed by

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the formation of the diacetate (III) on further acetylation of the monoacetate (II). In compound (III), the signal of the proton geminal to the acetoxy group is in a weaker field (δ 5.05 ppm). The fact that the secondary hydroxy group is comparatively readily acetylated excludes the C-11β position for it.

Oxidation of the monoacetate (II) by the Jones reagent yielded the oxowithanolide (IV). In the NMR spectrum of the diketone (IV), the signal of the 18-CH_3 group (1.12 ppm) is shifted downfield by 0.35 ppm in comparison with the corresponding signal for (II) (0.77 ppm), and the doublet of the 21-CH_3 group is shifted upfield ($\Delta = -0.11$ ppm). Such a shift usually indicates a keto group at C-12 [10]. Shifts of the same magnitude have been observed in the investigation of other 12-oxowithanolides – withanicandrin [11] and Nic-7 [12].

The R configuration at C-22 of the new withanolide was established in correspondence with the circular dichroism spectrum of compound (I), showing a positive Cotton effect at 253 nm ($\Delta\varepsilon$ +3.6).

The material present permits the assumption that withastramonolide (I) has the structure of 5α,12α,27-trihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2,24-dienolide.

EXPERIMENTAL

The UV spectrum was taken on a Specord UV-Vis spectrophotometer (C_2H_5OH), the IR spectrum on a UR-20 instrument (KBr), the mass spectra on an MKh-1303 instrument at an ionizing voltage of 40 V, the CD spectrum on a Jasco J-20 spectropolarimeter, and the NMR spectrum on a JNM-4H-100/100 MHz spectrometer in $CDCl_3$ with HMDS as internal standard (values recalculated to TMS in this paper).

For monitoring and identification in thin-layer chromatography (TLC) we used KSK silica gel with 5% of gypsum in the following systems: 1) benzene–ethyl acetate (1 : 1); 2) benzene–ethyl acetate (3 : 7); 3) chloroform–methanol (9 : 1); 4) acetone–chloroform (1 : 4); the chromatograms were revealed with a saturated chloroform solution of $SbCl_3$ at 110°C.

Isolation of Withastramonolide (I). Air-dry comminuted leaves of D. stramonium f. violacea (500 g) were extracted with methanol at room temperature. After evaporation of the solvent in vacuum the residue was treated with water (0.7 liter) and extracted with hexane to eliminate fats and pigments. The aqueous residue was repeatedly extracted with ether and then with chloroform. The ethereal fraction, after drying over Na_2SO_4, was evaporated. The green oily residue was dissolved in ethanol and was purified by passage of the solution through a column of Al_2O_3, the eluate being colorized with activated carbon. After the solvent had been distilled off, 0.70 g of a light green powder consisting of three substances (TLC, system 1) was obtained.

Similar treatment of the chloroform fraction yielded 0.40 g of combined withanolides.

The ethereal fraction contained mainly daturalactone with a small amount of withastramonolide and another withanolide. The chloroform fraction consisted of withastramonolide with traces of daturalactone and a minor amount of a third substance (because of its small amount it was not studied).

The mixture of withastramonolide with daturalactone was separated by means of preparative chromatography on a thin layer of silica gel with 5% gypsum fixed to glass (30 × 30 cm) in system 2. Separation of the ethereal and chloroform fractions yielded 0.125 g of crystalline withastramonolide (0.024% of the weight of the plant).

Withastramonolide (I). $C_{28}H_{38}O_7$, mp 264-266°C (from methanol), readily soluble in chloroform, sparingly in methanol, insoluble in ether; $[\alpha]_D^{25} +111.1 \pm 2°$ (c 1.89; chloroform); $\lambda_{max}^{C_2H_5OH}$ 225 nm (log ε 4.17; ε 14,904); ν_{max}^{KBr} 3300-3600, 1690, 1675 cm^{-1}. Mass spectrum, m/e (%): 486 (2, 3) M^+; 468 (23, 8), M – H_2O; 450 (14.2), M – $2H_2O$; 432 (7.1), M – $3H_2O$; 345 (23.8), M – 141; 327 (47), 345 – H_2O; 309 (21.4), 345 – $2H_2O$; 299 (38), M – 169 – H_2O; 281 (38), 299 – H_2O; 141 (57) lactone ring; 123 (100), 141 – H_2O. NMR spectrum δ: 0.77 (18-CH_3, s); 1.10 (21-CH_3, d), 1.17 (19-CH_3, s); 2.05 (28-CH_3, s); 3.05 (6-H, d); 3.35 (7-H, d); 4.05 (12-H, s); 4.42 (27-CH_2, s); 4.50 (22-H, m; 5.82 (2-H, dq), 6.60 (3-H, dq). CD spectrum (c 2.29; methanol): $\Delta\varepsilon = \pm 3.6$ (253 nm), $\Delta\varepsilon = -1.9$ (337 nm).

27-O-Acetylwithastramonolide (II) from (I). A solution of 60 mg of withastramonolide in a mixture of 1.2 ml of pyridine and 0.5 ml of acetic anhydride was left at room temperature. After 15 min, TLC in system 3 showed the presence of a new compound and the disappearance of the starting material. After the removal of the solvent in vacuum, the residue was twice recrystallized from aqueous methanol. This gave 52 mg of acicular crystals of 27-O-acetylwithastramonolide, $C_{30}H_{40}O_8$, mp 224-226°C, $[\alpha]_D^{28} +96.5 \pm 2°$ (c 1.47; chloroform); ν_{max}^{KBr} 3300-3600, 1690, 1710, 1735, 1235 cm^{-1}. Mass spectrum, m/e (%): 528 (1.8) M^+; 510 (28), M – H_2O; 492

(6.2), M − $2H_2O$; 468 (6.2) M − 60; 450 (28), 468 − H_2O; 432 (9.3), 468 − $2H_2O$; 345 (28), C-20−C-22 cleavage; 327 (38), 345 − H_2O; 309 (15.6), 327 − H_2O; 299 (37); 281 (37.5); 123 (100%). NMR spectrum, δ: 0.77 (18-CH_3, s); 1.10 (21-CH_3, d); 1.17 (19-CH_3, s); 2.0 ($OCOCH_3$, s); 2.05 (28-CH_3, s); 3.0 (6-H, d); 3.28 (7-H, d); 4.0 (12-H, s); 4.4 (22-H, m; 4.89 (27-CH_2, s); 5.78 (2-H, dq); 6.57 (3 H, dq).

12,27-Di-O-acetylwithastramonolide (III) from (II). A solution of 30 mg of (II) in 1 ml of pyridine and 0.5 ml of acetic anhydride was heated at 80°C for 2 h. After the usual working up, 30 mg of the diacetate (III) was obtained in the form of a pale amorphous powder which could not be crystallized; $[\alpha]_D^{28}$ +110.1 ± 3° (c 1.19; chloroform). NMR spectrum, δ: 0.85 (18-CH_3, s); 0.95 (21-CH_3, d); 1.16 (19-CH_3 s); 2.0 ($OCOCH_3$ s); 2.09 (28-CH_3, s) 3.05 (6-H, d); 3.32 (7-H, d); 4.4 (22-H, m); 489 (27-CH_2 s); 5.05 (12-H, s); 5.81 (2-H, dq); 6.58 (3-H, dq).

27-O-Acetyl-12-oxowithastramonolide (IV) from (II). At room temperature, 0.1 ml of the Jones reagent was added to a solution of 18 mg of the monoacetate (II) in 5 ml of acetone. After 10 min, TLC in system 4 showed the absence of the starting material. The reaction mixture was diluted with water and extracted with chloroform, and the extract was washed with water and dried over Na_2SO_4. After the solvent had been distilled off, recrystallization from methanol yielded 14 mg of 27-acetyl-12-oxowithastramonolide (IV), mp 220-222°C, ν_{max}^{KBr} 3515, 1735, 1710, 1690, 1235 cm^{-1}. Mass spectrum, m/e (%): 466 (100) M − 60; 448 (25), 466 − H_2O; 343 (50), C-20−C-22 cleavage; 325 (25), 343 − H_2O. NMR spectrum, δ: 0.99 (21-CH_3, d); 1.12 (18-CH_3 s); 1.24 (19-CH_3 s); 2.0 ($OCOCH_3$, s); 2.09 (28-CH_3, s); 3.08 (6-H, d); 3.40 (7-H, d); 4.45 (22-H, m); 4.9 (27-CH_2, s); 5.80 (2-H, dq); 6.60 (3-H, dq).

SUMMARY

A new withanolide called withastramonolide has been isolated from the leaves of Datura stramonium f. violaceae. It has the structure of 5α,12α,27-trihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2,24-dienolide.

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