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Isolation of 4β-Hydroxywithanolide E, a New Withanolide from *Physalis peruviana* L.

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From the leaves of *Physalis peruviana* L. (Solanaceae), a new withanolide, 4β-hydroxywithanolide E (I) has been isolated together with the known withanolide E (II). Chemical conversion of I into II confirmed the structure of I.

In the course of our search for medicinal resources from higher plants, we have examined the constituents of the leaves of *Physalis peruviana* L. (Solanaceae, South-American origin). Repeated chromatography of a methanolic extract of this plant on silica gel gave two crystalline compounds (I and II). Examination of their structures has shown that less polar compound is the known withanolide E (II)[2] and more polar one (I), a new compound, 4β-hydroxywithanolide E (I). Quite recently we learned from Prof. Lavie,[3] who accumulates a number of withanolides in his laboratory, that his group also obtained the compound (I) from the same plant grown in India and determined its structure mainly by comparison of its nuclear magnetic resonance (NMR) spectra with those of the known withanolides having similar substituents.[4] This information prompted us to report our chemical conversion of I to II, which clearly established the structure of I.

On the basis of infrared (IR), NMR and mass spectra, and physical constants, compound II was suggested to be identical with withanolide E which has already been isolated from *Withania somnifera* by Lavie and his co-workers.[2] Direct comparison of both compounds proved their identity.[5]

Another compound (I), $C_{28}H_{38}O_8$, was assumed to be a sort of withanolide from the elemental analysis and resemblance of its spectral data (see Experimental) to those withanolide E (II). On acetylation under mild conditions, compound I gave a monoacetate (III). Similarity of NMR spectrum of the acetate (III) to that of withaferin A acetate (IVa)[6] shows that these acetates should possess the same functional groups in the rings A and B. To confirm the presence of the allylic hydroxyl group on C-4, I was oxidized with Jones reagent to bring about a crystalline product (V), $C_{19}H_{22}O_5$, mp 257—259°, and an oily ketolactone (VI), $C_9H_{12}O_3$, ν_{max} cm^{-1}: 1716 and 1652. The former compound shows a two-proton singlet at δ 6.87, ν_{max} cm^{-1}: 1668 and λ_{max} nm (ε): 225.0 (12,600), suggesting the presence of ene-dione structure.[6] Furthermore, appearance of an IR band at ν_{max} cm^{-1}: 1743 (five-membered ring ketone) in V, and isolation of the ketolactone (VI) proved the presence of 17, 20-glycol system in I. The mass spectrum of compound I displays three peaks at *m/e* 484, 466, and 448, indicating the successive loss of three molecules of water from the structure (I). As the water molecule could not be eliminated from the secondary hydroxyl group on C-4 and hydrogen on C-3, there should be

1) Location: *a) Fukushima-ku, Osaka, 553, Japan; b) Koka-cho, Shiga, 520-34, Japan.*
2) D. Lavie, I. Kirson, and E. Glotter, *J. C. S. Chem. Comm.*, **1972**, 877.
3) D. Lavie, private communication.
4) I. Kirson, A. Abraham, S.S. Subramanian, and E. Glotter, *Phytochemistry*, **15**, 340(1976).
5) An authentic sample was furnished through the courtesy of Prof. Lavie.
6) *a)* D. Lavie, E. Glotter, and Y. Shvo, *J. Org. Chem.*, **30**, 1774 (1965); *b)* S.M. Kupchan, W.K. Anderson, P. Bollinger, R.W. Doskotch, R.M. Smith, J.A.S. Renauld, H.K. Schnoes, A.L. Burlingame, and D.H. Smith, *ibid.*, **34**, 3858 (1969).

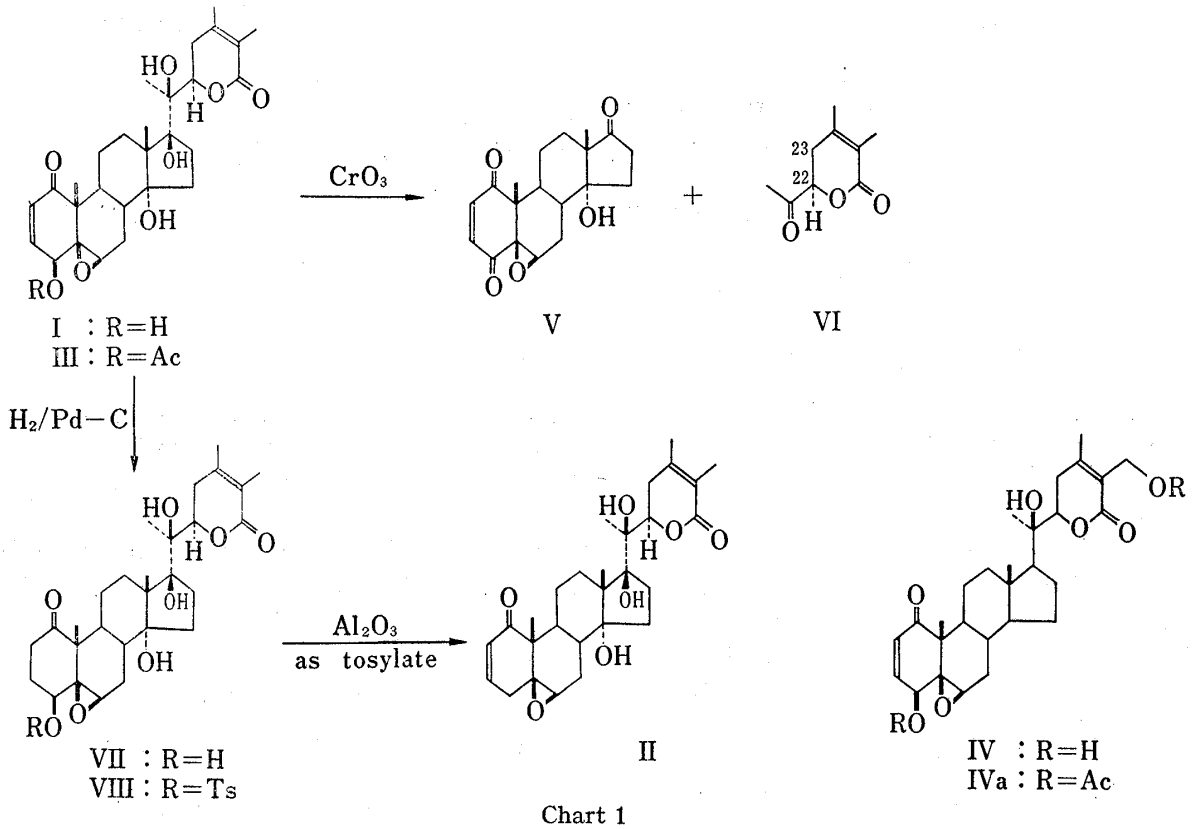

Chart 1

three tertiary hydroxyl groups in the molecule of I. Thus, the nature of all eight oxygen atoms in I is determined. Considering the co-existence of I with II in the plant, the last tertiary hydroxyl group was assumed to be on C-14. Then an attempt was made to convert I to II. Hydrogenation of I over Pd–C gave a 2,3-dihydroderivative (VII), which was esterified with tosyl chloride in pyridine. The tosylate (VIII) was treated with alumina[7] to give an olefinic compound, which was found to be identical with withanolide E (II) in all respects; the elimination of *p*-toluenesulfonic acid from VIII might form a double bond between C–3 and C–4 at the first stage, and then, it must migrate in a conjugated system.

The NMR spectrum of the 2,3-dihydro-compound (VII) shows H–4 signal at δ 3.50 (br. t, J=3 Hz) and H–6 signal at δ 3.17 (br. s). These values of chemical shifts accord well with those of 4β-hydroxy-5β,6β-epoxy-cholestane (δ 3.35 for H–4 and δ 3.16 for H–6)[8] and not with those of 4α-hydroxy-isomer (δ 3.98 for H–4 and 3.63 for H–6),[8] both compounds being synthesized by Lavie and his co-workers as the model compounds for determination of stereochemistry of withaferin A (IV). Thus, 4-hydroxyl group in I should have β-configuration.

The compound (I) showed a considerable life-span activity against L-1210 leukemia. The bioassay results will be published elsewhere.

Experimental

Isolation of 4β-Hydroxywithanolide E (I) and Withanolide E (II)——Dried and ground leaves (1.35 kg) of *Physalis peruviana* L., which was cultivated at our experimental farm in Aburahi Laboratories, were extracted with MeOH (3×20 liters) at room temperature. The combined methanolic extract (427 g) was washed with acetone (10 liters). The acetone-soluble portion (141 g) was washed with *n*-hexane and then triturated with 50% aqueous MeOH. The aqueous MeOH soluble portion (51 g) was chromatographed on a column of silica gel (Merck, 0.2—0.5 mm, neutral, 750 g). Eluate from MeOH–$CHCl_3$ (1: 99 and 2: 98) (4.14 g) was put on a column of silica gel (Merck, 0.06—0.2 mm, neutral, 120 g) and eluted with MeOH–$CHCl_3$ (1: 99) to yield an oily mixture (2.84 g). The mixture (1 g) was purified by preparative TLC on silica gel developed

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8) D. Lavie, Y. Kashman, and E. Glotter, *Tetrahedron*, **22**, 1103 (1966).

with EtOH–$CHCl_3$ (1: 9) to give withanolide E (II, *Rf* 0.52, 0.51 g): mp 167—168° (from EtOAc): $[\alpha]_D^{21.5}$ +121.7±1.6° (c=1.020, dioxane): UV λ_{max}^{EtOH} nm (ε): 226.5 (13,600): IR ν_{max}^{KBr} cm^{-1}: 3500—3390, 1715, 1667: NMR ($CDCl_3$) δ: 1.10 (3H, s, 21–CH_3), 1.25 and 1.42 (each 3H, s, 18– and 19–CH_3), 1.95 (6H, s, 26– and 27–CH_3), 3.18 (1H, br. s, H–6), 4.88 (1H, br. t, J=8 Hz, H–22), 6.00 (1H, dd, J=10, 2.5 Hz, H–2), 6.82 (1H, ddd, J=10, 5, 2.5 Hz, H–3): Mass Spectrum m/e: 486 (M^+), 468 ($M-H_2O$), 450 ($468-H_2O$), 432 ($450-H_2O$), 169, 152, 125, 109. *Anal.* Calcd. for $C_{28}H_{38}O_7 \cdot 1/2H_2O$: C, 67.85; H, 7.93. Found: C, 67.57; H, 8.00.

Elution of the first chromatography with MeOH–$CHCl_3$ (3: 97) yielded green crystals (2.44 g), which were decolored with small amount of activated charcoal. Recrystallization from EtOAc gave 4β-hydroxywithanolide E (I) as colorless prisms (2.01 g): mp 205—214°: $[\alpha]_D^{23}$ +107.0±1.5° (c=0.954, dioxane): UV λ_{max}^{EtOH} nm (ε): 219 (14700): IR ν_{max}^{KBr} cm^{-1}: 3540—3320, 1710, 1673: NMR [$CDCl_3$–CD_3OD (4: 1)] δ: 1.07 (3H, s, 21–CH_3), 1.37 and 1.43 (each 3H, s, 18– and 19–CH_3), 1.88 and 1.97 (each 3H, s, 26– and 27–CH_3), 3.25 (1H, br. s, H–6), 3.67 (1H, d, J=6 Hz, H–4), 4.85 (1H, dd, J=10, 7.5 Hz, H–22), 6.18 (1H, d, J=10 Hz, H–2), 7.02 (1H, dd, J=10, 6 Hz, H–3): Mass Spectrum m/e: 484 ($M-H_2O$), 466 ($484-H_2O$), 448 ($466-H_2O$), 169, 152, 125, 109. *Anal.* Calcd. for $C_{28}H_{38}O_8 \cdot 1/2$ EtOAc: C, 65.91; H, 7.74. Found: C, 66.11; H, 7.72.

Acetylation of I with Ac_2O and pyridine at room temperature gave an acetate (III) which could not be obtained in a crystalline form, but showed a single spot on a TLC [EtOH–$CHCl_3$ (1: 9)]: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460—3350, 1740, 1700: NMR ($CDCl_3$) δ: 1.07 (3H, s, 21–CH_3), 1.40 (6H, s, 18– and 19–CH_3), 1.88 and 1.92 (each 3H, s, 26– and 27–CH_3), 2.03 (3H, s, Ac), 3.28 (1H, br. s, H–6), 4.65 (1H, d, J=6.5Hz, H–4), 4.83 (1H, br. t, J=9 Hz, H–22), 6.23 (1H, d, J=10 Hz, H–2), 7.02 (1H, dd, J=10, 6.5 Hz, H–3).

Jones Oxidation of I——To a solution of 4β-hydroxywithanolide E (I, 156 mg) in acetone (2 ml) was added dropwise Jones reagent (5 drops) under stirring at room temperature. Stirring was continued for a further 10 min. Water was added to the reaction mixture, which was extracted with $CHCl_3$–EtOH (9: 1). Organic layer was washed with water, dried over Na_2SO_4 and evaporated to yield a pale yellow oil (152 mg). The oil was purified by preparative TLC on silica gel developed with EtOH–$CHCl_3$ (1: 9) to give two products of *Rf* 0.66 (63 mg) and of *Rf* 0.81 (21 mg). The compound of *Rf* 0.66 was recrystallized from ether to afford colorless prisms (V): mp 257—259°: $[\alpha]_D^{24}$ +135.0±1.7° (c=1.033, dioxane): UV λ_{max}^{EtOH} nm (ε): 225.0 (12,600): IR ν_{max}^{KBr} cm^{-1}: 3510, 3049, 1743, 1695, 1668, 1606: NMR ($CDCl_3$) δ: 1.02 and 1.42 (each 3H, s, 18– and 19–CH_3), 3.55 (1H, m, H–6), 6.87 (2H, s, H–2 and H–3): Mass Spectrum m/e: 330 (M^+), 312 ($M-H_2O$), 294, 284, 182, 151, 120 (base peak). *Anal.* Calcd. for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 69.18; H, 6.59.

The compound of *Rf* 0.81 was an oil (VI): $[\alpha]_D^{24}$ +153.6±1.6° (c=1.234, $CHCl_3$): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1716, 1652: NMR ($CDCl_3$) δ: 1.88 and 1.93 (3H each, with long-range couplings, 26– and 27–CH_3), 2.30 (3H, s, 21–CH_3), 2.58 (2H, m, H–23), 4.67 (1H, t, J=7 Hz, H–22): 2,4-dinitrophenylhydrazone: mp 206—208°. *Anal.* Calcd. for $C_{15}H_{16}O_6N_4 \cdot 1/4H_2O$: C, 51.06; H, 4.71; N, 15.87. Found: C, 51.11; H, 4.61; N, 16.20.

Catalytic Hydrogenation of I——4β-Hydroxywithanolide E (I, 33 mg) was hydrogenated in EtOH (2 ml) over 5% Pd–C (30 mg). One mole of hydrogen was absorbed within 3 min. Catalyst was filtered off and washed with EtOH. The filtrate and washings were combined and evaporated to yield a residue (33 mg), which was purified by preparative TLC on silica gel developed with a mixture of toluene–EtOAc–EtOH (2: 2: 1) to give colorless prisms (VII, *Rf* 0.62, 18 mg): mp 195—204° (from $CHCl_3$): $[\alpha]_D^{24.5}$ −16.3±0.5° (c=1.022, dioxane): UV λ_{max}^{EtOH} nm (ε): 228.0 (7,400): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630, 3570, 3380, 1705: NMR ($CDCl_3$) δ: 1.03 (3H, s, 21–CH_3), 1.28 and 1.43 (each 3H, s, 18– and 19–CH_3), 1.88 and 1.92 (each 3H, s, 26– and 27–CH_3), 3.17 (1H, br. s, H–6), 3.50 (1H, br., H–4), 4.85 (1H, br. t, J=8.5 Hz, H–22). *Anal.* Calcd. for $C_{28}H_{40}O_8 \cdot 1/2H_2O$: C, 65.47; H, 8.04. Found. C, 65.72; H, 8.08.

Tosylation of VII——To a solution of 2,3-dihydro-4β-hydroxywithanolide E (VII, 156 mg) in pyridine (0.5 ml) and $CHCl_3$ (2 ml) was added *p*-TsCl (307 mg) at room temperature and the mixture was kept for 3 days. Water was added to the mixture and stirred for 1 hr at room temperature. The mixture was extracted with $CHCl_3$, and the organic layer was washed successively with water, 10% HCl, water, 5% $NaHCO_3$ and water, and dried over Na_2SO_4. Evaporation of organic solvent gave a product (162 mg), which was purified by preparative TLC on silica gel developed with EtOH–$CHCl_3$ (1: 9) to afford colorless powder (VIII, *Rf* 0.63, 110 mg): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1705, 1600: NMR ($CDCl_3$) δ: 1.00 (3H, s, 21–CH_3), 1.13 and 1.40 (each 3H, s, 18– and 19–CH_3), 1.87 and 1.90 (each 3H, s, 26– and 27–CH_3), 2.43 (3H, s, aromatic CH_3), 3.15 (1H, br. s, H–6), 4.18 (1H, br., H–4), 4.63 (1H, br. t, H–22), 7.33 and 7.73 (2H each, A_2B_2q, J=8 Hz, aromatic H_4).

Treatment of Tosylate (VIII) with Alumina——A solution of tosylate (VIII, 185 mg) in warm C_6H_6 (4 ml) was absorbed to alumina (Merck, grade I, 5.4 g) and heated at 45° for 6 hr. The alumina was extracted with $CHCl_3$–MeOH (9: 1) and evaporation of the organic solvent gave an amorphous residue (167 mg). The residue was purified by preparative TLC developed with EtOH–$CHCl_3$ (1: 9) to give the starting material (VIII, *Rf* 0.52, 61 mg) and a product (*Rf* 0.42, 66 mg), colorless prisms (from EtOAc–CCl_4), mp 167°, $[\alpha]_D^{23.5}$ +114.6±3.0° (c=0.522, dioxane), which was identical with an authentic sample of withanolide E (II) (IR, NMR and mixed melting point determination).

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