STRUCTURES OF WITHAPERUVIN B AND C, WITHANOLIDES OF PHYSALIS PERUVIANA ROOTS[1]

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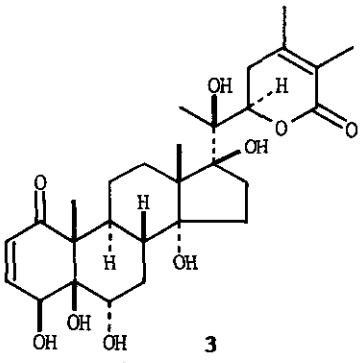
Abstract — Two minor withanolides, withaperuvin B and C, have been isolated from the roots of Physalis peruviana. Their structures have been elucidated as those represented by formulae **1** and **2**, respectively, based on chemical and spectral evidence.

In a previous publication,[2] the structure of withaperuvin, the major steroid of the roots of Physalis peruviana Linné (Solanaceae), was settled as **3** on the basis of spectral evidence and X-ray analysis of the derived androstane derivative (**4**). The present communication describes the structure determination of withaperuvin B and C, the two minor steroidal constituents isolated from the same plant source.



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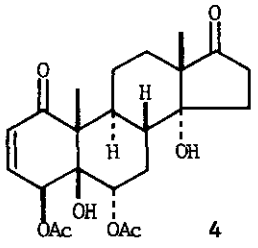

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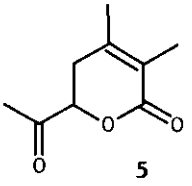
Withaperuvin B, m.p. 269-270°, $[\alpha]_D$ +215° (pyridine), was found to possess molecular weight 502 (FD-MS) which, together with the results of high resolution mass (M^+ at m/z 502.2532) and ^{13}C NMR spectroscopy (Table I), led to deduce its molecular formula $C_{28}H_{38}O_8$.

Withaperuvin B was recognized to be a close relative of withaperuvin (**3**) and both the substances were proved to have identical side chains and A/B ring substituents from the observations discussed in the sequel.

The IR spectrum of withaperuvin B displayed a characteristic band at 1700 cm^{-1} associated with an α,β-unsaturated δ-lactone, its mass spectrum showed peaks at m/z 169, 152 and 125, characteristic of 20-hydroxywithanolides, and on chromic acid oxidation it furnished the δ-lactone methyl ketone (**5**), identical with that obtained from withaperuvin (**3**) by the same treatment.



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The presence of an enone grouping in withaperuvin B was revealed from its UV absorption maximum at 222 nm (ε 11900), its IR band at 1665 cm^{-1} and also its ^1H NMR signals[3] for α- and β-hydrogens for an enone system at δ 5.86 and 6.50 (C-2 hydrogen and C-3 hydrogen) as an AB part of ABX system (J_{AB} 10 Hz).

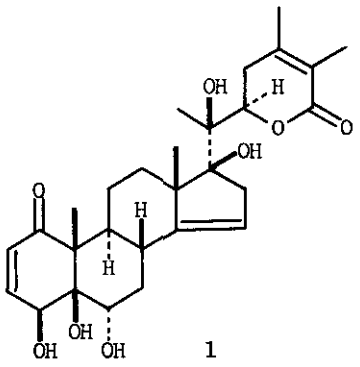
Table I. Carbon-13 shieldings in withaperuvin B, withaperuvin C and withaperuvin (δ)

	withaperuvin B (C_5D_5N)	withaperuvin C (C_5D_5N)	withaperuvin C (CD_3OD)	withaperuvin (C_5D_5N)
C-1	201.1 s	205.4 s	207.9	202.4 s
C-2	127.2 d	116.4 d	118.2	127.2 d
C-3	146.9 d	140.7 d	142.7	146.7 d
C-4	67.8 d	126.0 d	126.5	67.9 d
C-5	79.7 s	161.0 s	160.2	79.8 s
C-6	74.4 d	74.2 d	74.7	74.7 d
C-7	37.0 t	37.2 t	37.4	32.9 t
C-8	33.6 d	35.5 d	35.5	37.8 d
C-9	48.3 d	43.7 d	44.0	38.5 d
C-10	56.9 s	55.2 s	55.5	56.2 s
C-11	24.1 t	22.1 t	22.4	23.5 t
C-12	42.9 t	35.2 t	35.5	35.0 t
C-13	55.5 s	55.1 s	55.5	55.2 s
C-14	150.7 s	82.9 s	84.5	82.2 s
C-15	114.8 d	31.1 t	31.4	30.9 t
C-16	35.7 t	37.9 t	37.4	37.2 t
C-17	86.9 s	88.0 s	88.3	88.0 s
C-18	17.6 q	21.4 q	21.2	21.4 q
C-19	10.5 q	18.7 q	18.6	10.3 q
C-20	77.9 s	79.2 s	79.6	79.1 s
C-21	20.2 q	19.6 q	19.4	19.6 q
C-22	80.8 d	81.6 d	82.7	81.5 d
C-23	34.5 t	33.3 t	33.3	33.3 t
C-24	151.9 s	*	153.2	151.0 s
C-25	121.6 s	121.4 s	121.7	121.3 s
C-26	166.5 s	166.8 s	168.9	166.8 s
C-27	12.6 q	12.5 q	12.3	12.5 q
C-28	20.6 q	20.3 q	20.6	20.3 q

*not detected.

The observed couplings (J_{AX} 2 and J_{BX} 2 Hz) between the C-2 hydrogen signal at δ 5.86 and a carbinyl hydrogen signal at δ 5.15 and between the C-3 hydrogen signal at δ 6.50 and the carbinyl hydrogen signal at δ 5.15 demonstrated the location of a hydroxyl group at C-4. The identity of substitution pattern of the A and B rings in withaperuvin B and withaperuvin was further verified by the fact that the parameters of the ^1H NMR signals for the hydrogens at C-2, C-3, C-4, C-6 and C-19 *in withaperuvin B were essentially* identical with those of the corresponding hydrogens in withaperuvin (3) and that the CD curve of withaperuvin B ($[\theta]_{395}$ 0, $[\theta]_{350-324}$ ca. -3700, $[\theta]_{286}$ 0, $[\theta]_{232}$ +73300, dioxan) closely resembled that of withaperuvin ($[\theta]_{395}$ 0, $[\theta]_{349-317}$ -1900, $[\theta]_{295}$ 0, $[\theta]_{230}$ +75400, dioxan) in the entire span.

The ^{13}C NMR spectrum of withaperuvin B showed the existence of twenty-eight carbons (CH_3- x 5, $-CH_2$- x 5, >CH- x 3, >C< x 2, >CH-O x 2, >C-O x 3, -CH=CH- x 1, >C=CH- x 1, >C=C< x 1, >C=O x 2) (Table I). The resonances originating from the carbons in the A ring and the side chain of withaperuvin B were in agreement with those of the corresponding carbons in withaperuvin (3). The discrepancy in the chemical shifts for the carbons in the B, C and D rings may be rationalized by appropriate placement of the trisubstituted double bond, the presence of which in the molecule is indicated by ^1H NMR (1H broad singlet at δ 5.29) and ^{13}C NMR data (Table I). The most logical site for this double bond is the one between C-14 and C-15 as an unsaturation because this site is expected to be devoid of any influence on the resonances of the A ring carbons. The



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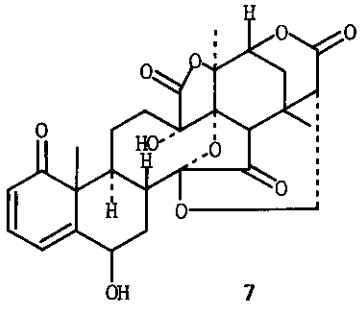
possibility of the presence of this ethylenic bond between C-7 and C-8 was excluded on the ground that no vicinal coupling is discernible between the signal for the carbinyl hydrogen at C-6 (δ 4.04) and that for the vinyl hydrogen (δ 5.29) in the ^{1}H NMR spectrum.

Based on the above data, withaperuvin B may be formulated as **1**, a direct evidence for which was secured by selective dehydration[4] of withaperuvin (**3**) to withaperuvin B (**1**).

Withaperuvin C, m.p. 190°, $[\alpha]_D$ -4.5° (pyridine), gave on acetylation the monoacetate (**6**), m.p. 155°. Withaperuvin C was revealed to have the composition $C_{28}H_{38}O_7$ based on the results of FD-mass spectral (M^+ at m/z 486), high resolution mass spectral (M^+-H_2O at m/z 468.2473) and ^{13}C NMR spectral data (CH_3- x 5, $-CH_2-$ x 6, >CH- x 2, >C< x 2, >CH-O x 2, >C x 3, -CH=CH- x 1, >C=CH- x 1, >C=C< x 1, >C=O x 2) (Table I).

The substance was proved to be a 17,20-dihydroxywithanolide by the observations of its diagnostic mass spectral peaks at m/z 169, 152 and 125, and its withanolide fingerprints in the ^{1}H NMR spectrum (1H double doublet contered at δ 4.87 and vinylic methyl singlets at δ 1.82 and 1.96) and finally by isolation of the δ-lactone methyl ketone (**5**) by chromic acid oxidation.

The IR spectrum of withaperuvin C showed in addition to the carbonyl absorption band at 1690 cm^{-1} (α,β-unsaturated δ-lactone), a band at 1660 cm^{-1} for a conjugated carbonyl group. The UV absorption maximum at 311 nm (ε 4370) displayed by withaperuvin C was found to match perfectly with the absorption characteristics (λ_{max} 312 nm, ε 4000) of physalin G (**7**).[5] These findings pointed to the presence of a 2,4-dien-1-one moiety in withaperuvin C. The same conclusion was also drawn from an analysis of the ^{1}H NMR spectrum of withaperuvin C which showed signals at δ 5.96, 7.03 and 6.17 for the vinyl hydrogens at C-2, C-3 and C-4, respectively, in an ABC pattern (J_{AB} 9, J_{BC} 6 Hz). The long-range coupling observed between the C-4 hydrogen signal at δ 6.17 and a carbinyl hydrogen signal at δ 4.60 (shifted to δ 5.55 in the acetate derivative (**6**)) indicated the presence of a hydroxyl at C-6. Withaperuvin C was thus proved to have a 6-hydroxy-2,4-dien-1-one system, the presence of which was further substantiated by comparison of the ^{1}H NMR spectrum ($CDCl_3$) of the acetate (**6**) with that of 6β-acetoxycholesta-2,4-dien-1-one (**8**)[6] when the chemical shifts and splitting patterns of the signals due to the hydrogens at C-2, C-3, C-4 and C-6 in both the spectra were found to coincide perfectly (δ 6.03, 6.93, 6.30 and 5.55 for the monoacetate (**6**) and δ 6.03, 6.90, 6.30 and 5.52 for 6β-acetoxycholesta-2,4-dien-1-one (**8**)).



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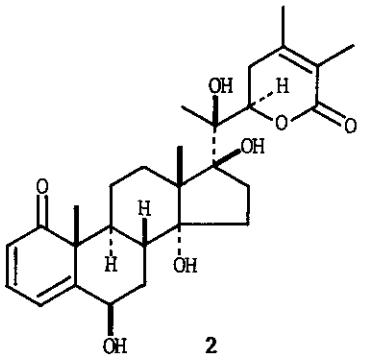
The configuration of the C-6 hydroxyl group was deduced to be β by the following ^{1}H NMR data: 1) the chemical shift of the C-19 methyl hydrogen signal (δ 1.48) of withaperuvin C was not compatible with that (δ 1.23, $CDCl_3$) of 6α-hydroxycholesta-2,4-dien-1-one (**9a**)[6] having the C-6α hydroxyl group, but was consistent with that (δ 1.42, $CDCl_3$) of its C-6 epimer (**9b**)[6] where the C-10 methyl and the C-6β hydroxyl were situated in the 1,3-diquasiaxial relationship which was further substantiated by the acetylation shift (Δδ 0.12 ppm) of the C-19 methyl hydrogen signal on passing from withaperuvin C (δ 1.48) to the monoacetate (**6**) (δ 1.36), 2) the splitting pattern (double doublet, J 3 and 3 Hz) of the C-6 carbinyl hydrogen signal in withaperuvin C showed the C-6 hydrogen to be quasiequatorial, and 3) an intramolecular nuclear Overhauser effect (17 %) was observed between the C-4 hydrogen signal (δ 6.17) and the C-6 hydrogen signal (δ 4.60).

The chemical shifts of the ^{13}C NMR signals attributed to the carbons in the D ring and the side chain of withaperuvin C are in accord with those of the corresponding carbons in withaperuvin (**3**), indicating that these structural moieties including the stereochemistry are identical in both

the substances.

The difference in the structural features in the A rings of the two molecules explains the observed discrepancy in the chemical shifts of the ^{13}C NMR signals for the carbons in the A, B and C rings. These data taken in conjunction with the facts that the CD curve of withaperuvin C ($[\theta]_{450}$ 0, $[\theta]_{380}$ +6400, $[\theta]_{348}$ 0, $[\theta]_{308}$ -50500, $[\theta]_{267}$ 0, $[\theta]_{250}$ +16100, dioxan) was almost overlapping on that of physalin G (**7**),[5] established the absolute stereochemistry of the environment of the A ring.

The above evidence permitted depicting the structure of withaperuvin C as **2**.



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NOTES AND REFERENCES

1) Part XXXII in the Tohoku University series on Steroids.
2) F. Frolow, A. B. Ray, M. Sahai, E. Glotter, H. E. Gottlieb and I. Kirson, J. Chem. Soc. Perkin Trans. I, 1981, 1029.
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