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Studies on the Constituents of Cistanchis Herba. II. Isolation and Structures of New Iridoids, Cistanin and Cistachlorin

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Two new iridoids, named cistanin and cistachlorin, were isolated together with four known compounds, D-mannitol, β-sitosterol, succinic acid and β-sitosterol-β-D-glucoside from the whole plant of *Cistanche salsa* (C. A. MEY.) G. BECK (Orobanchaceae). The structures of cistanin and cistachlorin were determined as I and II, respectively, on the basis of chemical and spectral data. Compounds I and II are non-glycosidic iridoids, possessing an ether structure of the type (C-1)–O–(C-10), and II also has a chlorohydrin moiety.

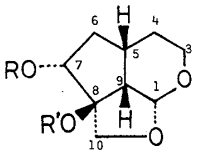
Keywords——*Cistanche salsa*; Cistanchis Herba; Orobanchaceae; parasitic plant; iridoid; cistanin; cistachlorin

Cistanche salsa (C. A. MEY.) G. BECK (Orobanchaceae) is a parasitic plant growing on the root of *Haloxylon ammodendron* (MEY.) BUNGE (Chenopodiaceae) and other desert plants, and the dried whole plants (called Cistanchis Herba) have been used as a staminal tonic under the name of Roucongrong in China[1] (Japanese name: Nikujuyou 肉蓯蓉).

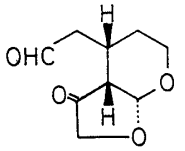
In the preceding paper,[2] we reported the isolation and structure elucidation of an iridoid glucoside, 8-epiloganic acid, and a monoterpene glucoside, 8-hydroxygeraniol-1-β-D-glucoside, as constituents of this crude drug. We now wish to report the isolation and structure elucidation of two new iridoids, I and II, isolated together with four known compounds, D-mannitol, β-sitosterol, succinic acid and β-sitosterol-β-D-glucoside, from this crude drug.

The whole plants were extracted with hot methanol and the residue of the extract, as a suspension in water, was partitioned with ethyl acetate and then with *n*-butanol. From the ethyl acetate-soluble fraction β-sitosterol and cistachlorin were obtained, while succinic acid, β-sitosterol-β-D-glucoside, cistanin and D-mannitol were obtained from the *n*-butanol-soluble fraction by repeated column chromatography on silica gel as described in the experimental section.

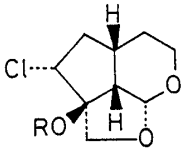
Cistanin (I) was obtained as colorless needles, $C_9H_{14}O_4$, mp 123—124 °C, $[\alpha]_D^{21}$ +62.6° (MeOH). Acetylation of I with acetic anhydride and pyridine under mild conditions afforded a monoacetate (Ia), $C_{11}H_{16}O_5$, mp 102—103 °C, $[\alpha]_D^{20}$ +131.0° ($CHCl_3$). On further acetylation under forcing conditions, Ia gave a diacetate (Ib), $C_{13}H_{18}O_6$, mp 58—59 °C, $[\alpha]_D^{20}$ +24.2° ($CHCl_3$), the infrared (IR) spectrum of which showed no hydroxyl group band. It follows therefore that I possesses two hydroxyl groups, and the difficulty of further acetylation of Ia is consistent with the presence of a tertiary hydroxyl group. The signal at δ 4.77 (1H, ddd, J=12, 6, 1.5 Hz) in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of I shifted to δ 5.02 in that of Ia, indicating the presence of a secondary hydroxyl group.



I : R=R′=H
Ia: R=$COCH_3$, R′=H
Ib: R=R′=$COCH_3$



Ic



II : R=H
IIa: R=$COCH_3$

Chart 1

The structure of I was clarified by the following spin decoupling experiments. In the ^{1}H-NMR spectrum of I, on irradiation of the doublet at δ 5.76 (1H, J=6 Hz) due to the acetalic H-1, the double doublet at δ 2.51 (1H, J=6, 9 Hz, H-9) changed into a doublet, and the reverse irradiation caused the doublet at δ 5.76 to change into a singlet and the multiplet at δ 2.38 (1H, H-5) to become deformed. The signals at δ 3.99 (1H, dd, J=10, 1.5 Hz) and at δ 5.22 (1H, d, J=10 Hz) seem to be due to the C-10 methylene protons from their coupling constants and splitting patterns. On irradiation of the double doublet at δ 3.99 due to H-10β, the doublet at δ 5.22 attributable to the H-10α and the double double doublet at δ 4.77 (1H, J=12, 6, 1.5 Hz, H-7) were changed into a singlet and a double doublet, respectively.

In the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of Ia, the C-7 signal at δ 83.2 showed a downfield shift by 4.0 ppm, and signals at C-6 (δ 34.1) and C-8 (δ 87.4) showed upfield shifts by 2.9 and 1.3 ppm, respectively, compared with those of I. Furthermore, in the spectrum of Ib, the C-8 signal at δ 92.9 showed a downfield shift by 5.5 ppm, and the signals at C-7 (δ 76.7), C-9 (δ 43.3) and C-10 (δ 68.1) showed upfield shifts by 6.5, 3.5 and 3.0 ppm, respectively, compared with those of Ia. These observations indicate that the secondary hydroxyl group is linked to the C-7 position and that the tertiary hydroxyl group is linked to the C-8 position.

Further, the existence of two free hydroxyl groups on neighboring carbon atoms in I is supported by the fact that periodic acid oxidation of I led to an aldehyde (Ic) possessing a five-membered ring ketone group [IR ($CHCl_3$) cm^{-1}: 1755 (C=O), 1725 (CHO). ^{13}C-NMR ($CDCl_3$) δ: 200.7 (CHO), 212.9 (C=O)].

Formation of Ic from I shows that cyclopentane ring cleavage occurs between C-7 and C-8, and consequently that the secondary hydroxyl group is attached to C-7 and the tertiary hydroxyl group to C-8.

In the ^{1}H-NMR spectrum of I, the signal of the H-10α proton appeared at δ 5.22 ppm (fairly low field), whereas in Ia the corresponding signal showed an upfield shift by 0.99 ppm compared with that of I. It follows therefore that the H-10α proton is subject to a paramagnetic shift due to the hydroxyl group at C-7, indicating that the proton and the hydroxyl group must be in a *cis*-relationship. Furthermore, in the ^{1}H-NMR spectrum of Ib, the signals of the H-7, H-9 and H-10β protons showed downfield shifts by 0.60, 0.24 and 0.24 ppm, respectively, compared with those of Ia. These shifts can be interpreted as being paramagnetic shifts[3] due to the ester carbonyl at C-8, indicating a *cis*-relationship between these protons and the tertiary hydroxyl group at C-8. Consequently the H-7, H-9 and H-10β protons and tertiary hydroxyl group were concluded to be β-oriented.

In the ^{1}H-NMR spectrum of I, the W form long-range coupling (J=1.5 Hz) between the H-7 and H-10β protons, as observed in the case of dihydrocatalpol hexaacetate,[4] is present. It follows therefore that the secondary hydroxyl group, linked to the C-7 position, is α-oriented (from a Dreiding stereo model) and the tertiary hydroxyl group, linked to the C-8 position, is β-oriented. The H-5 and H-9 protons have a *cis*-relationship, as shown by the coupling constant (J=9 Hz) between these protons.[5] The methyleneoxy group at C-8 links to

TABLE I. ^{1}H-NMR Chemical Shifts of I, Ia, Ib, Ic, II and IIa

Compound	H-1	H-3α H-3β	H-4α H-4β	H-5	H-6α H-6β	H-7	H-9	H-10α H-10β	$OCOCH_3$
I[a]	5.76 d (J=6)	4.18 ddd (J=12, 11, 2) 3.57 ddd (J=11, 4, 2)	1.28 br d (J=14) 1.83 m	2.38 m	1.96 ddd (J=12, 12, 6) 2.19 ddd (J=12, 12, 12)	4.77 ddd (J=12, 6, 1.5)	2.51 dd (J=9, 6)	5.22 d (J=10) 3.99 dd (J=10, 1.5)	
Ia[b]	5.38 d (J=6)	3.98 ddd (J=12, 11, 2) 3.58 ddd (J=11, 4, 2)	1.40 br d (J=14) 1.7—1.9 m	2.2—2.3 m	1.9—2.1 m	5.02 ddd (J=12, 6, 1.5)	2.38 dd (J=9, 6)	4.23 d (J=10) 3.46 dd (J=10, 1.5)	2.15 s
Ib[b]	5.44 d (J=6)	3.98 ddd (J=12, 11, 2) 3.56 ddd (J=11, 4, 2)	1.42 br d (J=14) 1.7—1.9 m	2.4—2.6 m	1.9—2.1 m	5.62 ddd (J=12, 6, 1.5)	2.62 dd (J=9, 6)	4.56 d (J=10) 3.70 dd (J=10, 1.5)	2.03 s 2.10 s
Ic[c]	5.37 d (J=6)	3.50 ddd (J=12, 11, 2) 3.82 ddd (J=11, 4, 2)	1.36 br d (J=14) 1.6—1.8 m	2.5—2.7 m	2.05 m	9.75 br s	2.85 dd (J=9, 6)	3.97 d (J=3)	
II[a]	5.72 d (J=6)	4.03 ddd (J=12, 11, 2) 3.54 ddd (J=11, 4, 2)	1.23 br d (J=14) 1.77 m	2.35 m	2.00 ddd (J=12, 12, 6) 2.13 ddd (J=12, 12, 12)	4.65 ddd (J=12, 6, 1.5)	2.52 dd (J=9, 6)	4.84 d (J=10) 3.99 dd (J=10, 1.5)	
IIa[b]	5.58 d (J=6)	3.98 ddd (J=12, 11, 2) 3.58 ddd (J=11, 4, 2)	1.42 br d (J=14) 1.7—1.9 m	2.3—2.6 m	1.9—2.2 m	4.72 ddd (J=12, 6, 1.5)	2.73 dd (J=9, 6)	4.56 d (J=10) 3.77 dd (J=10, 1.5)	2.10 s

δ ppm from TMS and J values in Hz.
a) Measured in C_5D_5N at 400 MHz.
b) Measured in $CDCl_3$ at 90 MHz.
c) Measured in acetone-d_6 at 90 MHz.

TABLE II. ^{13}C-NMR Chemical Shifts[a] of I, Ia, Ib, Ic II and IIa

Carbon No.	I	Ia[b]	Ib[b]	Ic[b]	II	IIa[b]
1	101.4	100.4	100.3	101.4	101.6	101.0
3	55.5	55.3	55.2	64.5	55.4	55.0
4	25.0	23.9	24.0	28.4	24.4	23.9
5	26.9	27.0	27.3	28.7	28.8	29.1
6	37.0	34.1	33.3	45.5	38.8	37.7
7	79.2	83.2	76.7	200.7	67.7	62.4
8	88.7	87.4	92.9	212.9	88.8	94.1
9	47.1	46.8	43.3	49.3	47.2	43.1
10	71.5	71.1	68.1	70.4	73.5	69.6
C=O		172.8	170.2			170.3
			170.2			
CH_3		20.8	21.0			21.7
			21.5			

a) δ ppm from TMS in C_5D_5N.
b) In $CDCl_3$.

C-1 forming a five-membered ether, so H-1 is β-oriented (from a Dreiding stereo model). Thus, the structure of cistanin was established to be I.

Cistachlorin (II) was obtained as colorless needles, $C_9H_{13}ClO_3$, mp 66—67 °C, $[\alpha]_D^{21}$ +59.1° (MeOH). The ^{1}H-NMR spectrum of II was very similar to that of I. Acetylation of II with acetic anhydride and pyridine afforded a monoacetate (IIa), $C_{11}H_{15}ClO_4$, mp 102—103 °C, $[\alpha]_D^{21}$ +26.4° ($CHCl_3$), which has no hydroxyl band in its IR spectrum. It follows therefore that II possesses a chlorine atom and one hydroxyl group. The ^{13}C-NMR spectrum of II revealed almost the same chemical shifts as those of I, except for the signal assignable to C-7. Furthermore, in the ^{13}C-NMR spectrum of IIa, the C-8 signal at δ 94.1 showed a downfield shift by 5.3 ppm, and the signals due to C-7 (δ 63.6), C-9 (δ 43.3) and C-10 (δ 69.7) showed upfield shifts by 4.1, 3.9 and 3.8 ppm, respectively, compared with those of II. All of the above data indicated that the hydroxyl group was located at C-8 and the chlorine atom at C-7. Therefore, II was assumed to be a chlorohydrin corresponding to I. Thus, the structure of cistachlorin was established to be II, possessing a 7α-chloro-8β-hydroxyl moiety.

In the iridoid series, a few compounds possessing a chlorohydrin moiety, for instance, linarioside,[6] eustoside,[7] *etc.* have been reported. Since cistachlorin from the water extract of the fresh plant material was identical with an authentic sample on thin layer chromatography (TLC) [$CHCl_3$–MeOH (10:1), *Rf* 0.51], we have confirmed that cistachlorin is a naturally occurring substance and not an artifact formed during the extraction and isolation procedure.

Iridoid derivatives similar to cistanin have only been reported as reaction products of catalpol,[4] aucubin[8] and genipin,[9] and so cistanin is the first compound of this type to be obtained as a natural product.

Experimental

Melting points were determined on a Mitamura micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded with a Hitachi 270-30 infrared spectrophotometer. ^{1}H-NMR spectra were recorded with a JEOL FX-90Q (90 MHz) or a JEOL JNM GX-400 (400 MHz) instrument. ^{13}C-NMR spectra were recorded with a JEOL FX-90Q spectrometer (22.5 MHz). Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; m, multiplet; br, broad). High resolution mass spectra (MS) and field desorption mass spectrometry (FD-MS) were measured with JEOL JMS D-300 and JEOL JMS-01-SG2 mass spectrometers, respectively. Gas liquid chromatog-

raphy (GLC) was run on a Shimadzu GC-4CM apparatus with a flame ionization detector. Kieselgel 60 F_{254} (Merck) prepared plates were used for TLC and detection was achieved by spraying 20% H_2SO_4 followed by heating.

Extraction and Isolation——The dried whole plants of *Cistanche salsa* (C. A. MEY.) G. BECK (10 kg, commercial crude drug produced in China) were chopped and extracted with MeOH (36 l × 2) under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was extracted with EtOAc and then with *n*-BuOH saturated with water. The EtOAc extract (97 g) was chromatographed on silica gel with $CHCl_3$–MeOH (50 : 1), and the eluate was separated into two fractions (Frs. 1 and 2). Fr. 1 was crystallized from MeOH to afford colorless needles (750 mg), mp 140—142 °C, IR ν_{max}^{KBr} cm^{-1}: 3300—3500 (OH). This product was identified as *β*-sitosterol by direct comparison (IR and GLC) with an authentic sample. GLC conditions (column, 1.5% OV-17, 3 mm × 1 m; column temp., 230 °C; carrier gas, N_2, 30 ml/min; t_R (min), 3.5). Fr. 2 was chromatographed on silica gel with *n*-hexane–acetone (3 : 1) to give cistachlorin (II) (150 mg).

The *n*-BuOH extract (120 g) was chromatographed on silica gel with a $CHCl_3$–MeOH solvent system to give three main fractions (Frs. 1, 2 and 3). Fr. 1, eluted with $CHCl_3$–MeOH (10 : 1), was rechromatographed on silica gel with $CHCl_3$–MeOH (10 : 1) to afford colorless needles (730 mg), mp 185—186 °C, IR ν_{max}^{KBr} cm^{-1}: 2760—2250, 1740, 1695 and a colorless powder (2.1 g), mp 281—283 °C, IR ν_{max}^{KBr} cm^{-1}: 3450, 1460, 1360 which were identified as succinic acid and *β*-sitosterol-*β*-D-glucoside, respectively, by direct comparisons (mixed mp and IR) with authentic samples. Fr. 2, eluted with $CHCl_3$–MeOH (6 : 1), was rechromatographed on silica gel using $CHCl_3$–acetone (1 : 1) to give cistanin (I) (540 mg). Fr. 3, eluted with $CHCl_3$–MeOH (2 : 1), was crystallized from MeOH to afford colorless needles (17.5 g), mp 167—168 °C; this product was identified as D-mannitol by direct comparison (mixed mp and IR) with an authentic sample.

Cistanin (I)——Colorless needles (from MeOH), mp 123—124 °C, *Anal.* Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found: C, 58.10; H, 7.61. $[\alpha]_D^{21}$ +62.6° (*c* = 1.33, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350, 1405, 1360, 1260, 1135, 1065, 1035, 950, 840. FD-MS (*m/z*): 187 ($M^+ + 1$), High resolution MS (*m/z*), Calcd for $C_9H_{14}O_4$: 186.0872. Found: 186.0890. MS *m/z* (%): 186 (M^+, 0.4), 156 (10.5), 155 (7.5), 138 (25.5), 112 (26.3), 110 (10.8), 96 (9.9), 95 (18.5), 85 (23.3), 84 (11.2), 83 (100.0), 82 (27.7), 81 (19.9). The ^{1}H-NMR and ^{13}C-NMR spectral data are given in Tables I and II, respectively.

Acetylation of I and Ia——Cistanin (I) (100 mg) was dissolved in pyridine–acetic anhydride (1 : 1) (2 ml) and the reaction mixture was allowed to stand for 2 h at room temperature, then poured into ice–water, and extracted with EtOAc. The EtOAc extract was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (100 : 1) to give the monoacetate (Ia) (85 mg) as colorless needles, mp 102—103 °C, $[\alpha]_D^{20}$ +131.0° (*c* = 1.25, $CHCl_3$), *Anal.* Calcd for $C_{11}H_{16}O_5$: C, 57.88; H, 7.07. Found: C, 57.65; H, 7.03. IR ν_{max}^{KBr} cm^{-1}: 3390, 1730, 1410, 1366, 1264, 1236, 1154, 1082, 1040, 946, 848. Cistanin monoacetate (Ia) (50 mg) was dissolved in pyridine–acetic anhydride (1 : 1) (1 ml) and the solution was allowed to stand overnight at 40 °C. The reaction mixture was treated in the same manner as described for I to give the diacetate (Ib) (43 mg) as colorless needles, mp 58—59 °C, $[\alpha]_D^{20}$ +24.2° (*c* = 1.78, $CHCl_3$), *Anal.* Calcd for $C_{13}H_{18}O_6$: C, 57.77; H, 6.71. Found: C, 57.82; H, 6.73. IR ν_{max}^{KBr} cm^{-1}: 1740, 1400, 1375, 1255, 1160, 1045, 955, 805.

The ^{1}H-NMR and ^{13}C-NMR spectral data for Ia and Ib are given in Tables I and II, respectively.

Periodic Acid Oxidation of I——Cistanin (I) (100 mg) was dissolved in 0.1 M aqueous periodic acid solution (10 ml) and the solution was allowed to stand for 2 h at room temperature. The reaction mixture was extracted with EtOAc. The EtOAc extract was concentrated and the residue was purified by column chromatography on silica gel with *n*-hexane–acetone (5 : 1) to give an aldehyde (Ic) (25 mg), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2860, 2730, 1755, 1725, 1372, 1055, 960, 915. MS *m/z*: 184 (M^+), 183 ($M^+ - 1$), 155 ($M^+ - CHO$), 154, 126. ^{1}H-NMR and ^{13}C-NMR spectral data are given in Tables I and II, respectively.

Cistachlorin (II)——Colorless needles (from EtOAc), mp 66—67 °C, $[\alpha]_D^{21}$ +59.1° (*c* = 0.17, MeOH), *Anal.* Calcd for $C_9H_{13}ClO_3$: C, 52.81; H, 6.40; Cl, 17.32. Found: C, 52.75; H, 6.34; Cl, 17.53. IR ν_{max}^{KBr} cm^{-1}: 3300, 1400, 1365, 1295, 1285, 1240, 1165, 1050, 945, 840. FD-MS (*m/z*): 205 ($M^+ + 1$). High resolution MS (*m/z*), Calcd for $C_9H_{13}ClO_3$: 204.0550. Found: 204.0519. MS *m/z* (%): 204 (M^+, 0.2), 203 (1.4), 174 (4.2), 139 (16.0), 138 (100.0), 121 (7.4), 109 (10.8). The ^{1}H-NMR and ^{13}C-NMR spectral data are given in Tables I and II, respectively.

Acetylation of II——Cistachlorin (II) (50 mg) was acetylated in the same manner as described for I to give the monoacetate (IIa) (40 mg) as colorless needles, mp 102—103 °C, $[\alpha]_D^{21}$ +26.4° (*c* = 0.95, $CHCl_3$), *Anal.* Calcd for $C_{11}H_{15}ClO_4$: C, 53.55; H, 6.13: Cl, 14.37. Found: C, 53.51; H, 6.23; Cl, 14.31. IR ν_{max}^{KBr} cm^{-1}: 1735, 1400, 1370, 1300, 1265, 1250, 1160, 1145, 1050, 950. The ^{1}H-NMR and ^{13}C-NMR spectral data are given in Tables I and II, respectively.

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