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Revised Structures of Inflexin and Related Diterpenoids

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The structure of inflexin isolated from *Rabdosia inflexa* (THUNB.) HARA was re-examined by means of ^{1}H two-dimensional correlated spectroscopy (^{1}H-COSY) and nuclear Overhauser effect (NOE) experiments, and was revised to **1** from the previously reported structure, **2**. The structures of the chemically correlated diterpenoids, inflexinol and inflexanin B were also revised to **3** and **4**, respectively.

Keywords——inflexin; inflexinol; inflexanin B; *Rabdosia inflexa*; ^{1}H-COSY; NOE; structure revision

Inflexin[2] has been isolated from *Rabdosia inflexa* (THUNB.) HARA[3] as the major constituent and reported to have the structure, *ent*-1α,3α,11β-trihydroxykaur-16-ene-6,15-dione 1,3-diacetate (**2**) by Kubo *et al.* on the basis of interpretation of the spectroscopic data and the results of some chemical reactions.[4] During the course of our studies on the minor constituents of *R. inflexa*, we re-examined the structure of inflexin and reached the conclusion that the structure should be revised to **1**. This paper deals with the structure revision of inflexin and chemically correlated diterpenoids, inflexinol[5] and inflexanin B.[6]

Inflexin has the *ent*-kaur-16-en-15-one structure as the basic skeleton from the reported spectroscopic data and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I). In addition to the basic skeleton, inflexin has a secondary hydroxy group [δ_H 4.22 (1H, d, J=6.3 Hz), OH; 3.99 (1H, ddd, J=4.8, 6.3 and 11.7 Hz), H_e], two secondary acetoxy groups [δ_H 1.96 and 2.12 (each 3H, s); 5.97 (1H, br d, J=5.0 Hz), H_b; 4.62 (1H, t, J=3.0 Hz), H_d], and an isolated ketone (δ_C 209.9) as judged from its proton-nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR spectra. The location of the three secondary carbinyl functional groups was examined by ^{1}H two-dimensional correlated spectroscopy (^{1}H-COSY). The ^{1}H-COSY spectrum of inflexin was shown in Fig. 1. The connectivities for H_a (17-H_l)→H_c (17-H_l)→H_g (13-H)→H_p (14β-H)→H_i (14α-H) were demonstrated by following the cross peaks through the line A. The connectivities for H_g→H_j (12α-H)→H_b (11-H)→H_k (9-H) were also demonstrated by following the cross peaks through the line B. Cross peaks between H_k and H_p, and H_m (12β-H) and H_p were also observed, which are ascribed to long-range coupling *via* W-interaction. Thus, an acetoxy group should be located at C-11. The configuration was presumed to be β as judged from the coupling pattern of H_b. The signal was coupled with H_j (12α-H), whereas the signal showed only small coupling with H_m (12β-H) and H_k (9-H) since the dihedral angles of H_b to H_m and H_k are near 90°. This presumption was further confirmed by the nuclear Overhauser effect (NOE) experiments described later. A ketone group was

TABLE I. ^{13}C-NMR Data for Inflexin (**1**) and Dehydroinflexin (**7**)[a]

Carbon	1	7	Carbon	1	7
1	74.9	208.1	12	37.5	38.6
2	32.3	38.6	13	36.4	36.1
3	78.2	80.9	14	36.6	36.8
4	35.9	36.5	15	205.6	204.6
5	58.1	60.2	16	148.5	147.9
6	209.9	207.8	17	115.1	116.0
7	50.2	49.3	18	26.3	26.0
8	54.9	53.4	19	21.8	21.7
9	60.2	50.5	20	14.2	18.0
10	51.0	58.1	$\underline{C}H_3CO$	21.3, 21.4	21.0, 21.3
11	70.3	69.2	$CH_3\underline{C}O$	170.1, 171.4	169.1, 169.8

a) The spectra were measured in $CDCl_3$ solution and the shifts are given in ppm (δ) relative to internal tetramethylsilane (TMS). Assignments were based on the results of ^{1}H-^{13}C-COSY and ^{1}H-^{13}C shift correlation by long-range coupling (COLOC) experiments.

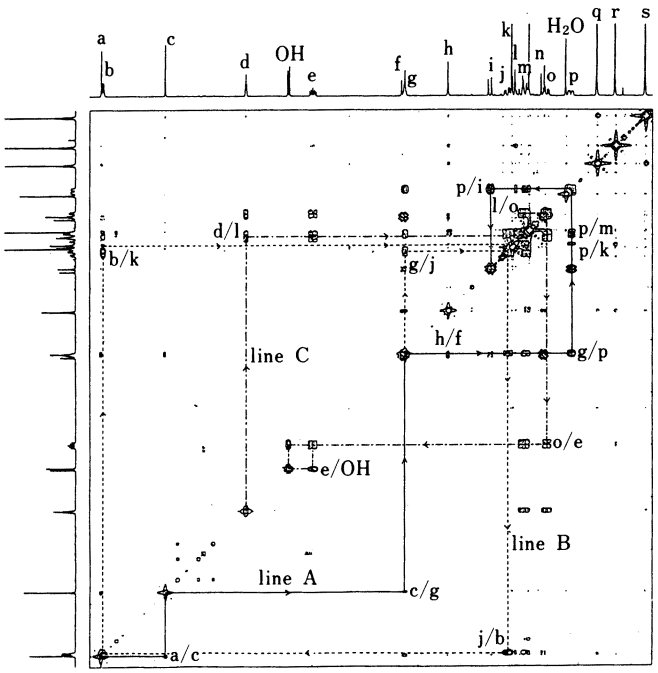


Fig. 1. ^{1}H-COSY Spectrum of Inflexin (**1**)

assigned to C-6 on the *ent*-kaurene nucleus since the signals due to a methine group (δ_H 2.72, br s, H_h, 5-H) and a methylene group [δ_H 3.14 (1H, dd, J=12.5 and 0.6 Hz), H_f, 7β-H; 1.83 (1H, d, J=12.5 Hz), H_n, 7α-H] which are adjacent to the carbonyl group were observed. The coupling between H_h and H_f was inferred from the observation of the cross peaks in the ^{1}H-COSY spectrum and was confirmed by decoupling experiments. The connectivities of the remaining groups [*i.e.* a methylene group (H_l and H_o), protons (H_e and H_d) on carbons having a hydroxy group and an acetoxy group] were demonstrated as $H_d \rightarrow H_l \rightarrow H_o \rightarrow H_e$ by following the cross peaks through the line C. Thus, the partial structure A was elucidated, which can be placed between C-1 and C-3 on the *ent*-kaurene nucleus. These connectivities were also supported by the ^{1}H-COSY spectrum (Fig. 2) of dehydroinflexin (**7**) obtained by Jones

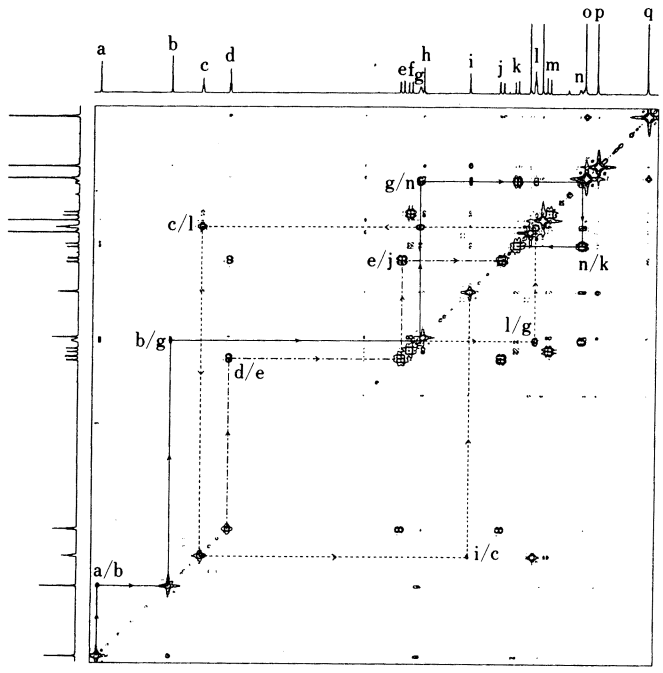


Fig. 2. ^{1}H-COSY Spectrum of Dehydroinflexin (**7**)

TABLE II. Results of NOE Experiments[a] for Inflexin (**1**)

Irradiated proton	Protons which showed NOE (%)
1-H	5-H (4.2), 9-H (13.7), 2β-H (5.3)
2α-H	3-H (5.0), 2β-H (12.4)
2β-H	3-H (4.2), 2α-H (29.2)
3-H	2α-H (6.2)
5-H	1-H (5.7), 7β-H (6.9), 9-H (12.3)
7α-H	7β-H (16.4)
7β-H	5-H (4.3), 7α-H (16.9)
9-H	1-H (11.6), 5-H (10.0), 11-H (4.4)
11-H	9-H (5.0)
12α-H	11-H (4.8)
14α-H	14β-H (15.0)
14β-H	14α-H (13.8)
17-H_1 (H_a)	17-H_1 (H_c) (31.6)
17-H_1 (H_c)	17-H_1 (H_a) (34.3)
18-H_3	3-H (8.5), 5-H (9.7)
19-H_3	3-H (8.6)
20-H_3	2α-H (27.5), 11-H (4.9), 14α-H (9.4), 19-H_3 (4.0)

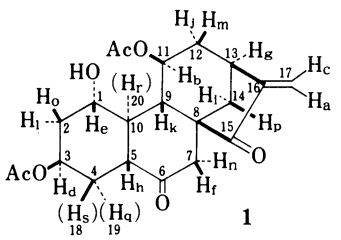
a) Experiments were performed on a solution of inflexin (**1**) (20 mg) in $CDCl_3$ (0.5 ml).

oxidation of inflexin. The connectivities $H_a \rightarrow H_b \rightarrow H_g \rightarrow H_n \rightarrow H_k$ and $H_g \rightarrow H_l \rightarrow H_c \rightarrow H_i$ were also confirmed as in the case of the ^{1}H-COSY spectrum of inflexin. The signals [δ_H 3.26 (1H, dd, J=3.3 and 14.3 Hz, H_e) and 2.34 (1H, dd, J=3.3 and 14.3 Hz, H_j)] which were assigned to the methylene protons adjacent to a ketone group showed cross peaks with H_d [δ_H 4.85 (1H, t, J=3.3 Hz)]. In this case, the signal (δ_H 2.63, 1H, br s, H_i) assigned to 9-H suffered a downfield

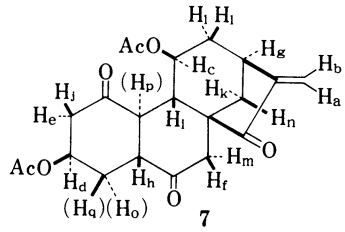
shift, suggesting that the newly formed carbonyl group in **7** and, consequently, a secondary hydroxy group in **1** are located at C-1.

In order to determine the locations of H_d and H_e, and the configurations of H_b, H_d and H_e as well as to confirm the stereochemistry of the ring system, extensive NOE experiments on inflexin were performed. Prior to the experiments, the assignments of three tertiary methyl groups (H_q, H_r and H_s) are necessary. In the ^{1}H-COSY spectrum, the two methyl groups (H_q and H_s) which gave ^{1}H singlets (δ_H 1.33 and 0.87) showed cross peaks with each other, indicating that they were a *gem*-dimethyl pair, *i.e.* methyl groups at C-4.[7] The remaining tertiary methyl group (δ_H 1.16; H_r) was assigned as 20-H_3. On irradiation at the frequency of H_r, an NOE (4%) was observed for H_q. Thus, H_q and H_s was assigned to 19-H_3 and 18-H_3, respectively. The results of NOE experiments on inflexin (**1**) are summarized in Table II. On irradiation at the frequencies of H_j and H_r, respectively, NOE's for H_b (4.8 and 4.9%, respectively) were observed. Thus, the acetoxy group at C-11 has a β-axial configuration. NOE's (8.5 and 8.6%, respectively) for H_d, another proton on a carbon having an acetoxy group, were observed on irradiation at the frequency of either H_s or H_q. On the other hand, an NOE for H_h (9.7%), those for H_e (5.7%) and H_k (12.3%), those for H_e (11.6%) and H_h (10.0%), and that for H_i (9.4%) were observed on irradiation at the frequency of H_s, H_h, H_k or H_r, respectively. These results not only showed the location of an equatorial hydroxy group at C-1 and an axial acetoxy group at C-3, but also confirmed the stereochemistry of the ring system. Thus, the structure of inflexin was determined as *ent*-1β,3α,11α-trihydroxykaur-16-ene-6,15-dione 3,11-diacetate (**1**). The difference between the revised structure and the previously reported structure is the location of a hydroxy group and an acetoxy group. Namely an axial acetoxy group at C-1 and an equatorial hydroxy group at C-11 in the old structure were interchanged, so that a β-axial acetoxy group and an α-equatorial hydroxy group are located at C-11 and C-1, respectively, in the revised structure. The abnormal downfield shift of H_b on the carbon having an acetoxy group was previously ascribed to the proximity of a hydroxy group at C-11 to the 1α-equatorial proton. On the other hand, the chemical shift can well be explained by the proximity of an equatorial hydroxy group at C-1 to H_b which takes an equatorial position at C-11 in the revised structure.

Inflexinol,[5] a minor constituent of *R. inflexa*, has been elucidated to have the structure (**5**) by comparison of its ^{1}H-NMR spectrum with that of inflexin and chemical conversion to inflexin acetate. The structure of inflexanin B,[6] another minor constituent of *R. inflexa*, was elucidated as **6** by comparison of its ^{1}H-NMR spectrum and conversion to inflexinol monoacetate. On the basis of the revised structure of inflexin (**1**), the structures of both inflexinol and inflexanin B should also be revised to **3** and **4**, respectively.



1



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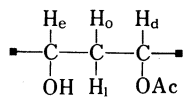
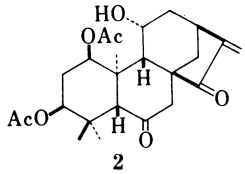


partial structure A



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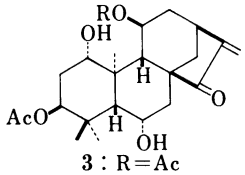
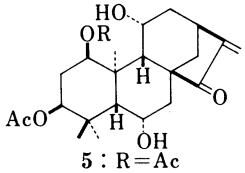


3 : R=Ac
4 : R=H



5 : R=Ac
6 : R=H

TABLE III. ^{1}H-NMR Data[a] for Inflexin (**1**) and Dehydroinflexin (**7**)

Proton	**1**	**7**
1-H	3.99 (ddd, 4.8, 6.3, 11.7)	
2α-H	2.02 (ddd, 3.0, 11.7, 15.0)	3.26 (dd, 3.3, 14.3)
2β-H	1.80 (ddd, 3.0, 4.8, 15.0)	2.34 (dd, 3.3, 14.3)
3-H	4.62 (t, 3.0)	4.85 (t, 3.3)
5-H	2.72 (br s)	3.06 (br s)
7α-H	1.83 (d, 12.5)	1.91 (d, 13.0)
7β-H	3.14 (dd, 0.6, 12.5)	3.18 (br d, 13.0)
9-H	2.09 (br s)	2.63 (br s)
11-H	5.97 (br d, 5.0)	5.10 (br t, 3.3)
12α-H	2.16 (ddd, 3.0, 5.0, 14.9)	2.03 (m)
12β-H	1.99 (m)	2.03 (m)
13-H	3.12 (m)	3.09 (m)
14α-H	2.33 (d, 12.4)	2.20 (d, 12.5)
14β-H	1.57 (dm, 12.4)	1.60 (br dd, 4.9, 12.5)
17-H_1	5.99 (t-like, 0.8)	6.04 (br s)
17-H_1	5.39 (t-like, 0.8)	5.38 (t-like, 0.8)
18-H_3	0.87 (s)	1.00 (s)
19-H_3	1.33 (s)	1.57 (s)
20-H_3	1.16 (s)	1.46 (s)
OAc	1.96, 2.12 (s)	1.97, 2.08 (s)
OH	4.22 (d, 6.3)	

a) The spectra were determined in $CDCl_3$ solution with TMS as an internal standard. Multiplicity and *J* values (in Hz) are given in parentheses.

Experimental

General procedures are the same as in the previous report[6] except for the NMR spectrometer. A Bruker AM-400 spectrometer (^{1}H, 400 MHz and ^{13}C, 100 MHz) was used for obtaining NMR data.

Inflexin (1)——Inflexin used in this report was previously isolated.[5] ^{1}H-NMR (see Table III); ^{13}C-NMR (see Table I).

Dehydroinflexin (7)——Jones reagent (0.35 ml) was added to a cooled solution of inflexin (**1**) (103 mg) dissolved in Me_2CO (10 ml) and the reaction mixture was stirred for 25 min. H_2O (30 ml) was added and the resulting precipitates were extracted with EtOAc (50 ml). The extract was washed with saturated aqueous NaCl, dried and evaporated *in vacuo* to give a residue (107 mg), which was purified by silica gel (10 g) column chromatography (solvent, $CHCl_3$) followed by preparative layer chromatography (solvent, $CHCl_3$–Me_2CO, 19 : 1, developed twice) to give dehydroinflexin (**7**) (97.2 mg). This substance was crystallized on addition of MeOH. mp 201—204 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725, 1715, 1640, 1370, 1250—1190, 1070, 1030. ^{1}H-NMR (see Table III). ^{13}C-NMR (see Table I). MS *m/z*: 430.1990 (M^+). Calcd for $C_{24}H_{30}O_7$: 430.1992.

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References and Notes

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