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The Structure of Shikokianidin, a Minor Component of *Isodon shikokianus* HARA

Takahiko ISOBE, Tadao KAMIKAWA, Isao KUBO, and Takashi KUBOTA
Faculty of Science, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka

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Shikokianidin from *Isodon shikokianus* Hara is formulated as structure (**3**) on the basis of chemical and spectroscopic evidence.

The isolation of two bitter principles, oridonin (**1**) and shikokianin (**2**) from the extract of *Isodon shikokianus* Hara has already been reported.[1] From the less polar fractions of the extract we have obtained a new, non-bitter diterpene, designated shikokianidin (**3**), $C_{26}H_{34}O_9$, mp 218—219°C, $[\alpha]_D$ $-109°$. Shikokianidin showed hydroxylic, acetoxy and 6-membered ketonic absorptions in the infrared spectrum (ν_{max} 3450, 1745, 1725, 1260, and 1230 cm^{-1}). Its NMR spectrum (Fig. 1) showed signals attributable to two tertiary C-methyl groups (δ 1.10 and 1.41), three acetyl groups (δ 1.95, 2.02, and 2.12), and a hydroxyl group (δ 4.33, s, exchangeable with D_2O). These spectroscopic data account for eight oxygen atoms out of nine of those of shikokianidin. In addition, there is a three-proton multiplet at *ca.* δ 4.9 which could be assigned to three –CH(OAc)–protons. The lack of a signal for –CH(OH)– in the NMR spectrum confirmed the tertiary nature of the free hydroxyl group. Other significant features of the NMR spectrum of (**3**) include signals arising from ≡C–CH_2–O– (δ 4.23, dd, J=2 and 10 Hz; δ 4.84, dd, J=1.5 and 10 Hz) and a terminal methylene group (δ 5.07, dd, J=1.5 and 3 Hz; δ 5.43, t, J=3 Hz). From analogy with congeners the former group probably constitutes a hemiacetal system and the latter is involved in the D ring.

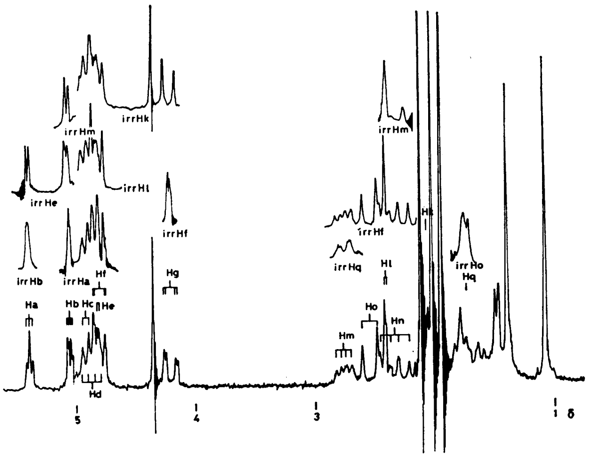
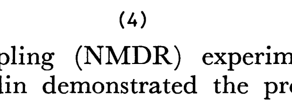


Fig. 1. The NMR spectrum of shikokianidin (**3**).



(4)

Spin decoupling (NMDR) experiments performed on shikokianidin demonstrated the presence of structural unit (**4**). On irradiation of the H_e proton located at δ 4.85 (d, J=1.5 Hz), the triplet at δ 5.43 (H_a) collapsed to a doublet (J=3 Hz), showing the removal of allylic coupling and the retention of geminal coupling (J_{gem}=3 Hz). When the H_a proton was irradiated the H_e proton appeared as a singlet with a small splitting (J<1 Hz), indicative of a neighbouring quaternary centre, and the H_b proton (δ 5.07) collapsed to a doublet ($J\simeq$1 Hz). On the other hand, when the H_m proton located at δ 2.77 (q, J=5 and 9 Hz) was irradiated, the H_b proton collapsed to a sharp doublet ($J_{a,b}$=3 Hz) and the H_n proton at δ 2.36 (q, J=9 and 16 Hz) to a doublet ($J_{n,p}$=16 Hz). Conversely,

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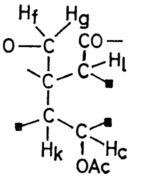
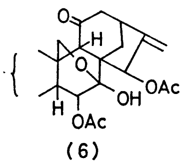
[1] T. Kubota and I. Kubo, This Bulletin, **42**, 1778 (1969).

irradiation at δ 1.75 (H_q) resulted in the collapse of the H_m proton signal to a broad double doublet ($J_{m,n}=9$, $J_{b,m}=2$ Hz), and irradiation of the H_o proton at δ 2.56 (d, $J_{o,q}=13$ Hz) resulted in the collapse of the multiplet at δ 1.75 to a doublet ($J_{m,q}=5$ Hz). The results suggest that the H_m proton is coupled to H_b, H_n, and H_q and the latter two protons to H_p and H_o, respectively, and the dihedral angles between H_m and H_p, H_m and H_o are both close to 90°. The magnitude of the geminal coupling constant between H_n and H_p ($J=16$ Hz) suggests that these two protons are adjacent to a carbonyl group.[2] Furthermore, NMDR experiments of **3** afforded evidence for the presence of structural unit (**5**).



(5)

On irradiation of the H_l proton at δ 2.42 (d, $J=1.5$ Hz), the double doublet pattern for H_f proton (δ 4.84, $J=1.5$ and 10 Hz) was reduced to a doublet ($J=10$ Hz) showing the removal of long-range coupling through a W-configuration and the retention of geminal coupling. Irradiation at the centre of the double doublet for H_f proton resulted in the collapse of the H_l proton signal to a singlet and the H_g proton signal to a doublet ($J=2$ Hz). The chemical shift of the H_l proton is indicative of the presence of a neighbouring carbonyl group.[3] On irradiation at δ 2.06 (H_k proton), the double doublet pattern for H_g proton (δ 4.23, $J=2$ and 10 Hz) was reduced to a doublet ($J=10$ Hz) and the double doublet pattern for H_c proton (δ 4.93, $J=5$ Hz) was reduced to a singlet, showing the removal of both long-range coupling through a W-configuration and vicinal coupling. Thus expansion of the part structures **4** and **5** to **6** would be admissible if we assume that shikokianidin has ent-kaurane skeleton with a hemiacetal ring. Further evidence to support this part structure was provided by acid treatment of shikokianidin.



(6)

Treatment of **3** with 2N hydrochloric acid gave a seco-aldehyde **7**, $C_{24}H_{32}O_8$. The NMR spectrum of the seco-aldehyde showed the presence of two tertiary C-methyl groups (δ 1.07 and 1.33), two acetyl groups (δ 1.90 and 2.09), a hydroxyl group (δ 4.56, exchangeable with D_2O), two olefinic protons conjugated with an aldehyde (δ 6.00, s; δ 6.33, d, $J=1.5$ Hz) and an aldehyde proton (δ 9.51, s). (see Fig. 2, where the lower part of the spectrum is omitted).

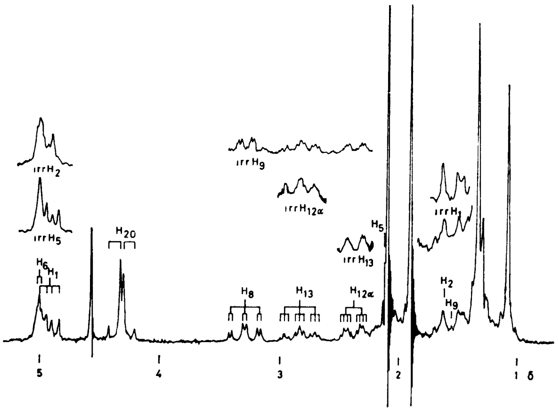
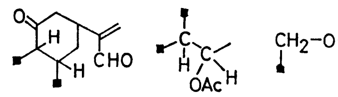


Fig. 2. The NMR spectrum of seco-aldehyde (**7**).

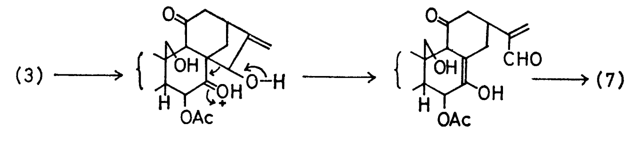
NMDR experiments demonstrated the presence of the part structure (**8**).



(8)

The results lead to assignment of structure **7** to the seco-aldehyde except for the position of one secondary acetoxy group in ring A. From biogenetic grounds, it is most reasonable to place this acetoxy group at position C_1 or C_3. The NMR spectrum of **7** showed a one proton quartet at δ 4.91 ($J=6$ and 10 Hz) and a one proton doublet at δ 4.99 ($J=4$ Hz), the latter being assigned to H_7 (on irradiation at δ 2.14 this collapsed to a singlet), and the former to $H_{1\beta}$ or $H_{3\beta}$ (on irradiation at δ 1.63 this collapsed to a doublet $J=10$ Hz). The axial nature of this proton was evident from the large axial-axial coupling ($J=10$ Hz) and small axial-equatorial coupling ($J=6$ Hz). The splitting pattern of this proton is quite similar to that of the C_1 proton of shikokianin (**2**).[4] A possible mechanism for this transformation would be a hydrolysis of the allylic acetate with concomitant opening of the hemiacetal as demonstrated in trichokaurin,[5] followed by reverse aldol reaction and re-cyclization to hemiacetal.

Catalytic reduction of shikokianidin gave dihydro-



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[2] T. Takahashi, *Tetrahedron Lett.*, **1964**, 565.

[3] S. Forsen and T. Norin, *ibid.*, **1964**, 2845.

[4] The original assignment of the NMR spectrum of **2** was incomplete and some conflicting results were reported.[1] The correct assignment is as follows; two tertiary *C*-methyl protons (δ 1.16 and 1.18), two acetyl protons (δ 1.91 and 2.11), two hydroxyl protons (δ *ca.* 4.7, C_7–OH and δ 5.93, d, $J=12$ Hz, C_6–OH; both exchangeable with D_2O), $H_{12\beta}$ (δ 2.82, q, $J=4$ and 15 Hz), $H_{12\alpha}$ (δ 2.40, q, $J=9$ and 15 Hz), $H_{14\alpha}$ (δ 2.84, d, $J=13$ Hz), H_{13} (δ 3.07, q, $J=4$ and 9 Hz), H_6 (δ 4.01, q, $J=10$ and 12 Hz), $\equiv C–CH_2–O–$ (δ 4.11, d, $J=10$ Hz with small splitting; δ 4.44, q, $J=2$ and 10 Hz), H_1 (δ 4.74, q, $J=5$ and 10 Hz), H_{11} (δ 4.86, t, $J=4$ Hz) and two olefinic protons (δ 5.50 and 6.00).

[5] E. Fujita, T. Fujita, M. Shibuya, and T. Shingu, *Tetrahedron*, **25**, 2517 (1969).

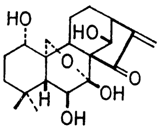
shikokianidin (**9**), $C_{26}H_{36}O_9$. The NMR spectrum of **9** showed the presence of a secondary methyl group (δ 0.71, d, $J=7$ Hz) and a -CH(OAc)- proton (δ 4.86, d, $J=10$ Hz). NMDR experiments demonstrated that the proton attached to the secondary methyl group and the proton of the type -CH(OAc)- are vicinal (on irradiation at δ 2.51, both the secondary methyl and the -CH(OAc)- proton signals collapsed to singlets). Since the hydrogenation of the C_{15} double bond is assumed to occur from the less hindered side of the molecule, the newly introduced secondary methyl is presumably β. The dihedral angle between the protons H_{15} and H_{16} is assumed to be 0° from the coupling constant ($J=10$ Hz). Thus the acetoxy group at C_{15} can be assigned as β.

Treatment of **9** with methanolic ammonia gave trisdeacetyldihydroshikokianidin (**10**), $C_{20}H_{30}O_6$, with an IR frequency (1710 cm^{-1}) compatible with a cyclohexanone. The NMR spectrum (in $CDCl_3$–C_5D_5N) showed signals for one secondary (δ 0.96, d, $J=7$ Hz) and two tertiary (δ 1.07 and 1.11) *C*-methyl groups, three -CH(OH)- protons (δ 3.90, q, $J=7$ and 9 Hz, H_1; δ 4.57, d, $J=11$ Hz, H_{15}; δ 4.78, q, $J=3$ and 8 Hz, H_6) and $\equiv$C–CH_2–O– protons (δ 4.60, q, $J=$ 1 and 12 Hz; δ 5.95, d, $J=12$ Hz).

From analogy with the congeners a tentative assignment of stereochemistry as shown in **3** can now be extended to shikokianidin. On examination of a molecular model, the proton H_5 and the proton H_9 are in close proximity (both axial). When the proton at C_5 was irradiated, NOE enhancement was observed in the integrated area of H_9 signal (15%). Another relative stereochemistry may now be deduced from a consideration on the NMR data of shikokianidin. As already discussed, the acetoxy groups at C_1 and C_{15} can be assigned as α and β, respectively. The C_6 proton gives rise to a doublet ($J=4$ Hz). This suggests an equatorial disposition with coupling to an adjacent axial C_5 proton (the ring B takes a boat conformation). The C_{13} proton showed a spectrum assumed to be the X part of an ABX system ($J_{12\alpha,13}=$ 9 Hz, $J_{14\beta,13}=5$ Hz, with small allylic coupling). This suggests that the C_{13} proton is equatorial and the dihedral angles between H_{13} and $H_{12\beta}$, and H_{13} and $H_{14\alpha}$ are both 90°. The stereochemistry of the seco-aldehyde produced by an acid-treatment of **3** can now be formulated as **7**. It is anticipated that ring C takes a strain-free chair form and that the ring junctures take the most stable *trans-anti-trans* configuration. The C_8 proton (δ 3.28) showed a six-line spectrum with coupling constants $J_{8,9}=J_{8,14\beta}=12$ Hz, $J_{8,14\alpha}=3$ Hz. The low-field shift of this signal is attributable to the proximity of the hemiacetal oxygen atom.[6] The C_{13} proton (δ 2.82) showed a nine-line spectrum with coupling constants $J_{12\beta,13}=J_{13,14\beta}=13$ Hz, $J_{12\alpha,13}=J_{13,14\alpha}=$ 4 Hz.

Shikokianidin shows a positive CD. However, no similar compounds are available for a comparative study.

Finally, we have confirmed the proposed structure by relating **3** to shikokianin (**2**), which in turn relates to nodosin.[7] Lithium aluminum hydride reduction of **3** gave a mixture of three reduction products. One of the reduction products was deacetylshikokianidin. The other two products were isomeric pentaols (**11**) and (**12**). On the other hand, the reduction of shikokianin (**2**) with lithium aluminum hydride gave pentaol (**11**), the latter proving to be identical with one of the pentaols derived from **3**. Thus the structure of shikokianidin including the absolute configuration has been established.



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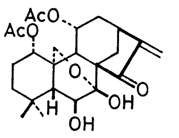

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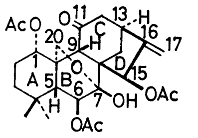

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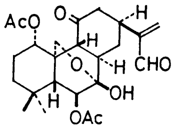

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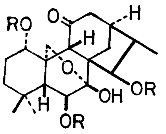
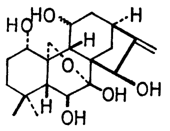


(9) R=Ac
(10) R=H



(11) 11α-OH
(12) 11β-OH

Experimental

NMR spectra were determined on a JEOL PS-100 (100 MHz) spectrometer in deuterochloroform solutions, unless otherwise stated. IR spectra were recorded on Nujol mull with a Japan Spectroscopic IR-S spectrophotometer. CD data were obtained in chloroform solution with a Jasco ORD/UV-5 (equipped with CD attachment). Column chromatography was performed with Mallinckrodt silicic acid.

Isolation. The dried leaves of *Isodon shikokianus* Hara were extracted with ether. The crude extract was treated with activated charcoal, concentrated and chromatographed in ether over silicic acid. Elution with the same solvent gave shikokianidin, shikokianin, and oridonin in this order. The less polar fractions were collected and recrystallised from ethanol to give pure shikokianidin (**3**), mp 218—219°C, $[\alpha]_D$ −109° (pyridine). Found: C, 63.79; H, 7.14%. Calcd for $C_{26}H_{34}O_9$: C, 63.66; H, 6.99%, ν_{max} 3450, 1745, 1725, 1260 and 1230 cm^{-1}, λ_{max}^{EtOH} 301 nm (ε 77).

Acid-treatment of Shikokianidin (3). Shikokianidin (320 mg) was heated in refluxing methanol (15 ml) containing 2 N hydrochloric acid (15 ml) for 3 hr. On cooling, crystals were separated and recrystallised from ethanol to give the seco-aldehyde (**7**), mp 268—269°C. Found: C, 64.24; H, 7.31%. Calcd for $C_{24}H_{32}O_8$: C, 63.98; H, 7.61%, ν_{max} 3400, 1730, 1700 and 1240 cm^{-1}.

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[6] L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London (1969), p. 80.

[7] E. Fujita, T. Fujita, and M. Shibuya, *Tetrahedron Lett.*, **1966**, 3153; E. Fujita, T. Fujita, and M. Shibuya, *Chem. Pharm. Bull.* (Tokyo), **16**, 506 (1968).

*Dihydroshikokianidin (**9**).* Shikokianidin was hydrogenated in ethanol over 10% palladium-charcoal. The crude product was recrystallised from dilute ethanol to give dihydroshikokianidin (**9**) as colorless prisms, mp 178—180°C, Found: C, 63.33; H, 7.43%. Calcd for $C_{26}H_{36}O_9$: C, 63.40; H, 7.37%, ν_{max} 3480, 1730, 1260, and 1230 cm^{-1}, δ 0.71 (3H, d, J=7 Hz, *sec* CMe), 1.09 and 1.39 (each 3H, *tert* CMe), 1.98, 2.00, and 2.14 (each 3H, OAc), 2.34 (1H, d, J=1 Hz, H_9), 4.22 (1H, dd, J=2 and 10 Hz, H_{20}), 4.34 (1H, s, OH), 4.83 (1H, dd, J=1 and 10 Hz, H_{20}), 4.87 (1H, d, J=10 Hz, H_{15}), *ca.* 4.9 (1H, m, H_1).

*Trisdeacetyldihydroshikokianidin (**10**).* Dihydroshikokianidin (100 mg) was dissolved in ammonia saturated methanol (20 ml). After the mixture had been left standing for 23 days at room temperature, it was concentrated to dryness under reduced pressure. The residue was chromatographed over preparative plates to give trisdeacetyldihydroshikokianidin (**10**), (29 mg), mp 268—271°C (dec) (from acetone–light petroleum). Found: C, 65.50; H, 8.30%. Calcd for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25%, ν_{max} 3360, 1710, and 1180 cm^{-1}, δ ($CDCl_3$-C_5D_5N) 0.96 (3H, d, J=7 Hz, *sec* CMe), 1.07 and 1.11 (each 3H, *tert* CMe), 2.25 (1H, s, H_9), 2.72 (1H, dd, J=1 and 6.5 Hz, H_5), 3.90 (1H, q, J=7 and 9 Hz), 4.57 (1H, d, J=11 Hz, H_{15}), 4.60 (1H, dd, J=1 and 12 Hz, H_{20}), 4.78 (1H, dd, J=3 and 8 Hz, H_6), 5.47 (broad OH), 5.95 (1H, d, J=12 Hz, H_{20}).

Reduction of Shikokianidin with LAH. Shikokianidin (50 mg) was refluxed with lithium aluminum hydride (100 mg) in ether (20 ml) for 3 hr. The excess of the reagent was decomposed with ethyl acetate and the resulting emulsion was washed with dilute hydrochloric acid and then with water. Drying and evaporation of the solution gave a residue which was purified by tlc ($CHCl_3$–MeOH, 9:1) to yield deacetylshikokianidin and the mixture of pentaols (A) and (B). Pentaol (A) was identified as ent-5β,20-epoxykaur-16-ene-1β,6α,-7α,11β,15α-pentaol (**11**) by tlc (solvent system: $CHCl_3$–MeOH, 9: 1; AcOEt-CH_2Cl_2, 7: 3; CH_2Cl_2–Me_2CO, 1: 1).

Reduction of Shikokianin with LAH. Similarly, shikokianin (300 mg) was reduced with lithium aluminum hydride to give pentaol (**11**), mp 253—255°C (dec) (from methanol–chloroform), Found: C, 64.18; H, 8.28%. Calcd for $C_{20}H_{30}O_6\cdot 1/2H_2O$: C, 63.98; H, 8.32%, ν_{max} 3300, 1650, and 1565 cm^{-1}.

We are grateful to Dr. K. Kuriyama, Shionogi Research Laboratory, for the CD measurements.