Two New C(20)-Oxygenated *ent*-Kaurene Diterpenoids from *Isodon henryi*

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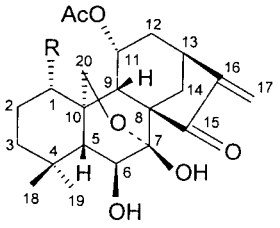
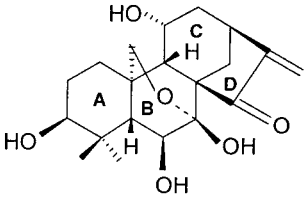
Two new C(20)-oxygenated *ent*-kaurene diterpenoids, taibaihenryiins A (**1**) and B (**2**), along with five known compounds, shikokianin (**3**), longikaurin D, 2α-hydroxyursolic acid, longikaurin F, and lasiodin, were isolated from the EtOH extract of the leaves and tender branches of *Isodon henryi* (HEMSL.) KUDO. The structures of the new compounds were determined as 11α-acetoxy-7,20-epoxy-1α,6β,7β-trihydroxy-*ent*-kaur-16-en-15-one (**1**) and 7,20-epoxy-3β,6β,7β,11α-tetrahydroxy-*ent*-kaur-16-en-15-one (**2**) on the basis of detailed spectroscopic analyses.

Introduction. – Some plants of the genus *Isodon* which have been used for gastrointestinal disorder, antitumor, and antiphlogistic agents in Chinese traditional medicine, contain diterpenoids with various biological activities such as antitumor, antibacterial, and inhibitory activities on the respiration of rat mitochondria and for insect growth [1]. Previous studies of the chemical constituents of *Isodon henryi* (HEMSL.) KUDO led to the isolation of more than ten diterpenoids [2–6].

In a further study of the minor diterpenoid constituents of this species, we have now isolated from the same plant collected from Taibai Mountain two new C(20)-oxygenated *ent*-kaurene diterpenoids, taibaihenryiins A (**1**) and B (**2**), together with five known compounds. The structure elucidation of the new compounds **1** and **2** was achieved by spectroscopic methods, including 1D- and 2D-NMR.



1 R=OH
3 R=AcO



2

Results and Discussion. – Taibaihenryiin A (**1**) was obtained as colorless crystals from $CHCl_3$, and the molecular formula $C_{22}H_{30}O_7$ was determined by FAB-MS (*m/z* 407 ($[M+1]^+$)) and HR-FAB-MS. The UV and IR spectra of **1** show characteristic absorption bands for a five-membered-ring ketone conjugated with an exocyclic methylene group (231 nm; 1708, 1636 cm^{-1}). The ^{13}C-NMR (*Table 1*) and ^{1}H-NMR

spectra of **1** exhibit the signals for three Me, five CH_2, and six CH groups, four quaternary C-atoms, two olefinic C-atoms, one ketone C=O, and one ester C=O. Moreover, ^{1}H-NMR (*AB* system at δ 4.52 and 4.02) and ^{13}C-NMR spectra (hemiacetal group at δ 94.8) are consistent with a 7,20-epoxy-*ent*-kaur-16-en-15-one skeleton carrying 1 AcO and 3 OH groups [7]. ^{1}H,^{1}H-COSY Data (*Table 2*) and comparison with shikokianin (**3**) and 7,20-epoxy-*ent*-kaurene-type diterpenoids allowed us to assign to taibaihenryiin A (**1**) the structure 11α-acetoxy-7,20-epoxy-1α,6β,7β-trihydroxy-*ent*-kaur-16-en-15-one.

The ^{13}C-NMR data of **1** are very similar to those of shikokianin (**3**), except for the absence of 1 AcO group. Comparison of their ^{13}C-NMR data indicate that the difference between **1** and **3** is only at C(1). This suggests that two OH groups are at C(6) and C(7), and an AcO group is at C(11). On the other hand, the upfield shift of C(1), from δ 76.4 in **3** to 73.5 in **1**, indicates that OH–C(1) in **1** has replaced the AcO group in **3**. These results are further confirmed by the ^{1}H,^{1}H-COSY data of **1** (*Table 2*).

Previous studies of the NMR spectra of 7,20-epoxy-*ent*-kaurene-type diterpenoids show that ring A adopts the chair conformation, ring B has a boat conformation, ring D is in an envelope conformation, ring C is in either a chair or a boat conformation depending on the substituents at ring C, and OH–C(6) and OH–C(7) are β-positioned [8]. In compound **1**, ring C should be in a boat conformation and AcO–C(11) is α-positioned considering the coupling constants of H_α–C(12) (δ 2.38, *dd*, J = 15.8, 9.1 Hz), H_α–C(13) (δ 3.11, *dd*, J = 9.1, 4.5 Hz), and H_β–C(11) (δ 5.34, *t*, J = 4.3 Hz). The OH–C(1) of **1** should be in an α-orientation as judged from the coupling of H_β–C(1) (δ 5.89, br. *d*, J = 11.6 Hz) and H_α–C(2).

Taibaihenryiin B (**2**) was obtained as colorless crystals from MeOH, and the molecular formula $C_{20}H_{28}O_6$ was determined by FAB-MS and elemental analysis. The ^{13}C-NMR (*Table 1*) and ^{1}H-NMR data indicate that compound **2** possesses two Me,

Table 1. *^{13}C-NMR Data of* **1**–**3**[a])

	1	**2**	**3**
C(1)	73.5 (*d*)	39.0 (*t*)	76.4 (*d*)
C(2)	29.7 (*t*)	34.0 (*t*)	25.0 (*t*)
C(3)	39.0 (*t*)	74.4 (*d*)	38.8 (*t*)
C(4)	33.4 (*s*)	34.1 (*s*)	33.4 (*s*)
C(5)	57.8 (*d*)	60.9 (*d*)	57.8 (*d*)
C(6)	74.6 (*d*)	72.7 (*d*)	74.6 (*d*)
C(7)	94.8 (*s*)	95.7 (*s*)	94.8 (*s*)
C(8)	58.3 (*s*)	59.3 (*s*)	58.2 (*s*)
C(9)	53.8 (*d*)	54.3 (*d*)	53.3 (*d*)
C(10)	42.5 (*s*)	42.5 (*s*)	41.5 (*s*)
C(11)	69.5 (*d*)	66.5 (*d*)	69.6 (*d*)
C(12)	38.0 (*t*)	39.1 (*t*)	37.7 (*t*)
C(13)	33.5 (*d*)	34.2 (*d*)	33.4 (*d*)
C(14)	25.8 (*t*)	26.6 (*t*)	25.8 (*t*)
C(15)	208.1 (*s*)	211.3 (*s*)	207.2 (*s*)
C(16)	151.9 (*s*)	154.1 (*s*)	151.5 (*s*)
C(17)	118.6 (*t*)	116.1 (*t*)	119.4 (*t*)
C(18)	33.7 (*q*)	28.9 (*q*)	33.8 (*q*)
C(19)	22.7 (*q*)	22.8 (*q*)	22.8 (*q*)
C(20)	64.5 (*t*)	65.1 (*t*)	64.8 (*t*)
MeCO	170.8 (*s*), 21.8 (*q*)		170.3 (*s*), 169.8 (*s*), 22.0 (*q*), 21.6 (*q*)

[a]) The data of **1** and **3** were recorded in $CDCl_3$, those of **2** in (D_6)DMSO.

Table 2. *^{1}H,^{1}H-COSY Data of* **1** *and* **2**[a])

	1	**2**
H_α–C(1)	–	H_β–C(1), H_α–C(3), H_b–C(20) (H_α–C(2), H_β–C(2))
H_β–C(1)	H_a–C(20), (OH_α–C(1))	H_α–C(1), H_α–C(3), H_a–C(20)
H_α–C(2)	H_β–C(2), (H_α–C(3)), H_β–C(3))	H_β–C(2), (H_α–C(1), H_α–C(3))
H_β–C(2)	H_α–C(2), (H_α–C(3), H_β–C(3))	H_α–C(2) (H_α–C(1), H_α–C(3))
H_α–C(3)	H_β–C(3) (H_α–C(2), H_β–C(2))	H_α–C(1), H_β–C(1), Me(18), Me(19), (H_α–C(2), H_β–C(2), OH_β–C(3))
H_β–C(3)	H_α–C(3), (H_α–C(2), H_β–C(2))	–
H_β–C(5)	(H_α–C(6))	H_β–C(9) (H_α–C(6))
H_α–C(6)	(H_β–C(5))	(H_β–C(5), OH_β–C(6))
H_β–C(9)	(H_β–C(11))	H_β–C(5), H_α–C(6), H_α–C(12) (H_β–C(11))
H_α–C(11)	–	–
H_β–C(11)	(H_β–C(9), H_β–C(12), H_α–C(12))	(H_β–C(9), H_α–C(12), H_β–C(12), OH_α–C(11))
H_α–C(12)	H_β–C(12), (H_β–C(11), H_a–C(13))	H_β–C(9) (H_β–C(11), H_β–(12), H_α–C(13))
H_β–C(12)	H_α–C(12) (H_β–C(11))	H_α–C(12), H_β–C(14), OH_α–C(11) (H_β–C(11), H_α–C(13))
H_α–C(13)	H_a–C(17) (H_b–C(17), (H_α–C(12), H_α–C(14))	H_a–C(17), H_b–C(17), (H_α–C(12), H_β–C(12), H_α–C(14), H_β–C(14))
H_α–C(14)	H_β–C(14) (H_α–C(13))	H_β–C(14 (H_α–C(13))
H_β–C(14)	H_α–C(14)	H_β–C(12), H_α–C(14) (H_α–C(13))
H_a–C(17)	H_α–C(13), H_b–C(17)	H_α–C(13), H_b–C(17)
H_b–C(17)	H_α–C(13), H_a–C(17)	H_α–C(13) H_a–C(17)
Me(18)	n. o.[b])	H_α–C(3)
Me(19)	n.o.[b])	H_α–C(3)
H_a–C(20)	H_β–C(1)	H_β–C(1), H_b–C(20)
H_b–C(20)	n.o.[b])	H_α–C(1), H_a–C(20)
OH_α–C(1)	(H_β–C(1))	–
OH_β–C(3)	–	(H_α–C(3))
OH_β–C(6)	–	(H_α–C(6))
OH_β–C(7)	n.o.[b])	n.o.[b])
OH_α–C(11)	–	H_β–C(12) (H_β–C(11))

[a]) The data of **1** were recorded in $CDCl_3$, those of **2** in (D_6)DMSO. Three-bond correlations are indicated in parentheses. [b]) n.o. indicates no clear correlations.

five OH_2, and six CH groups, four quaternary C-atoms, two olefinic C-atoms, and one ketone C=O. The UV and IR spectra of **2** show characteristic absorption bands for a five-membered-ring ketone conjugated with an exocyclic methylene group (234 nm; 1709, 1641 cm^{-1}). The ^{1}H-NMR (*AB* system at δ 4.38 and 3.84) and ^{13}C-NMR spectra (quaternary C at δ 95.7 (*s*)) of **2** suggest a 7,20-epoxy-*ent*-kaur-16-en-15-one diterpenoid structure with 4 OH groups. ^{1}H,^{1}H-COSY Data (*Table 2*) and comparison with similar compounds finally allowed us to assign to taibaihenryiin B (**2**) the structure 7,20-epoxy-3β,6β,7β,11α-tetrahydroxy-*ent*-kaur-16-en-15-one.

Two OH groups of **2** are attached in β-positions at C(6) and C(7), as deduced from the comparison of its spectral data with those of 7,20-epoxy-*ent*-kaurene-type diterpenoids [8]. The locations of the other 2 OH groups can be assigned on the basis of the ^{1}H-NMR and ^{1}H,^{1}H-COSY data (*Table 2*) of **2**. The signal at δ 3.61 (*t*, $J = 3.3$, 1 H) is attributed to H–C(3) as deduced from its ^{1}H,^{1}H-COSY correlation with Me(18) and Me(19) (δ 1.04 and 1.03, 2 s). H–C(3) (δ 3.61) also correlates with OH–C(3) (δ 5.88, *d*, $J = 3.1$ Hz), suggesting the

presence of an OH group at C(3). The signal at δ 3.12 is assigned to H–C(13); it shows ^{1}H,^{1}H-COSY cross-peaks with the signals of CH_2(14) (δ 2.91 and 1.87) and CH_2(12) (δ 2.30 and 1.55). The signals of CH_2(12) correlate with that of H–C(11) (δ 4.17). Moreover, OH–C(11) (δ 5.49) correlates with H–C(11) (δ 4.17) and H_β–C(12) (δ 1.55). Thus, **2** possesses a structural unit CH_2–CH–CH_2–CH in ring C and an OH group at C(11). The relative configuration of OH–C(3) and OH–C(11) are established as being β and α, repectively, as judged from the coupling constants of H–C(3) (δ 3.61, *t*, J = 3.3 Hz) and H–C(11) (δ 4.17, br. *d*, J = 3.2 Hz).

Compounds **3** and three other isolated diterpenoids were identified as shikokianin (=(1α,6β,7β,11α)-1,11-bis(acetoxy)-7,20-epoxy-6,7-dihydroxykaur-16-en-15-one[1])), longikaurin D (=(4α,6β,7β,11α)-18-acetoxy-7,20-epoxy-6,7,11-trihydroxykaur-16-en-15-one[1])), longikaurin F (=(4α,6β,7β,11α)-11,18-bis(acetoxy)-7,20-epoxy-6,7-dihydroxykaur-16-en-15-one[1])), and lasiodin (=(1α,6β,7β,14*R*)-1-acetoxy-7,20-epoxy-6,7,14-trihydroxykaur-16-en-15-one[1])), respectively, and a fifth compound as 2α-hydroxyursolic acid (=(2α,3β)-2,3-dihydroxyurs-12-en-28-oic acid[1])), as established by comparison of the physical and ^{13}C-NMR data with reported ones [9–13].

Experimental Part

General. M.p.s: X_4 micro melting-point apparatus; uncorrected. Optical rotations: *Jasco 20C* polarimeter. UV spectra: *Hitachi U-2000* spectrophotometer; MeOH soln.; $\lambda_{max.}$ (log ε) in nm. IR spectra: *Nicolet Avatar-360 FT-IR* spectrometer; KBr pellets; in cm^{-1}. NMR spectra: *Bruker Avance-DMX500* instrument; chemical shifts δ in ppm as internal standard; rel. to $SiMe_4$ (=0 ppm), J in Hz. FAB-MS: *ZAB-HS* instrument (data system MASPEC II) and *Autospec 3000* instrument (high resolution); in *m*/*z*. Elemental analysis: *Perkin-Elmer PE2400* CHN elemental analyzer.

Plant Material. The plant material was collected from Taibai Mountain, Shaanxi Province, P. R. China, in August, 1997. It was identified as *Isodon henryi* (HEMSL.) KUDO. A voucher specimen (SNU 97-08-02, Li) was deposited in the herbarium of the Department of Biology, Shaanxi Normal University.

Extraction and Isolation. The dried powdered leaves and tender branches of *Isodon henryi* (5.0 kg) were extracted with 95% EtOH (3 × 15000 ml) at r.t. for 7 d. After evaporation, the residue was partitioned in H_2O and extracted with petroleum ether (3 × 3000 ml) and AcOEt (3 × 3000 ml). The AcOEt extract (122 g) was subjected to CC (silica gel) (2 kg, 200–300 mesh), $CHCl_3$/Me_2CO 10:0 → 0:10). The eluate obtained with $CHCl_3$/Me_2CO 8:2 was further submitted to repeated CC ($CHCl_3$/acetone 9:1 → 8:2) to give **3** (1.65 g, 0.033%), *longikaurin D* (2.18 g, 0.044%), *2α-hydroxyursolic acid* (0.64 g, 0.013%), *longikaurin F* (84 mg, 0.0017%), *lasiodin* (74 mg, 0.0015%), and **1** (61 mg, 0.0012%), subsequently. The eluate obtained with $CHCl_3$/Me_2CO 7:3 was purified by CC: **2** (55 mg, 0.0011%).

Taibaihenryiin A (=*(1α,6β,7α,11α)-11-Acetoxy-7,20-epoxy-1,6,7-trihydroxykaur-16-en-15-one*[1]); **1**) Colorless crystals from $CHCl_3$. M.p.: 174–176°. $[\alpha]_D^{25} = -32.1$ (c = 0.56, $CHCl_3$). UV: 231 (3.56). IR: 3436, 3340, 3288, 2910, 1708, 1636, 1465, 1366, 1274. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.89 (br. *d*, J = 11.6, H_β–C(1)); 1.24 (overlap, H_α–C(2)); 1.44 (*m*, H_β–C(2)); 1.59 (*d*, J = 3.5, H_α–C(3)); 1.25 (*m*, H_β–C(3)); 1.47 (*m*, H_β–C(5)); 3.55 (*dd*, J = 11.0, 5.8, H_α–C(6)); 1.56 (overlap, H_β–C(9)); 5.34 (*t*, J = 4.3, H_β–C(11)); 2.38 (*dd*, J = 15.8, 9.1, H_α–C(12)); 1.79 (*dd*, J = 15.8, 5.1, H_β–C(12)); 3.11 (*dd*, J = 9.1, 4.5, H_α–C(13)); 2.16 (*dd*, J = 12.5, 4.5, H_α–C(14)); 2.85 (*d*, J = 12.5, H_β–C(14)); 6.01 (br. *s*, H_a–C(17)); 5.50 (br. *s*, H_b–C(17)); 1.17 (*s*, Me(18)); 1.16 (*s*, Me(19)); 4.52, 4.02 (*AB* ('*dd*'), J = 9.3, H_a–C(20), H_b–C(20), 2.06 (*s*, AcO); 3.94 (br. *t*, J = 9.7, OH_β–C(1)); 2.18 (*s*, OH_β–C(7)). ^{1}H,^{1}H-COSY: *Table 2*. ^{13}C-NMR: *Table 1*. FAB-MS: 407 ($[M+1]^+$). HR-FAB-MS: 406.4672 (M^+, $C_{22}H_{30}C_7^+$; calc. 406.4694).

Taibaihenryiin B (=*(3β,6β,7α,11α)-7,20-Epoxy-3,6,7,11-tetrahydroxykaur-16-en-15-one*[1]); **2**). Colorless crystals from MeOH. M.p. 251–253°. $[\alpha]_D^{25} = -72.4$ (c = 0.23, DMSO). UV: 234 (3.49). IR: 3309, 2931, 1709, 1641, 1457, 1395, 1177, 1060. ^{1}H-NMR (500 MHz, (D_6)DMSO): 1.51 (*m*, H_α–C(1)); 1.18 (*d*, J = 6.7, H_β–C(1)); 1.49 (br. *d*, J = 13.6, H_α–C(2)); 1.27 (*dd*, J = 13.6, 2.0, H_β–C(2)); 3.61 (*t*, J = 3.3, H_α–C(3)); 1.37 (*dd*, J = 10.5, 3.0, H_β–C(5)); 3.66 (*dd*, J = 10.8, 7.2, H_α–C(6)); 1.62 (*m*, H_β–C(9)); 4.17 (br. *d*, J = 3.2, H_β–C(11)); 2.30 (*dd*, J = 15.1, 8.1, H_α–C(12)); 1.55 (*dd*, J = 15.1, 5.1, H_β–C(12)); 3.12 (*dd*, J = 9.6, 4.3, H_α–C(13)); 2.91 (br. *d*, J = 11.5, H_α–C(14)); 1.87 (*dd*, J = 11.5, 4.3, H_β–C(14)); 5.74 (*s*, H_a–C(17)); 5.44 (*s*, H_b–C(17)); 1.04, 1.03 (2*s*, Me(18), Me(19)); 4.38, 3.84 (*AB* ('*dd*'), J = 9.6, H_a–C(20)), H_b–C(20)); 5.88 (*d*, J = 3.1, OH_β–C(3)); 5.96 (*d*, J = 10.9, OH_β–C(6)); 6.25 (*s*, OH_β–C(7)); 5.49 (*d*, J = 2.7, OH_α–C(11)). ^{1}H,^{1}H-COSY: *Table 2*. ^{13}C-NMR:

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[1]) Systematic name based on the kaurane skeleton used by *Chem. Abstr.*; according to IUPAC, this skeleton is *ent*-kaurane [14].

Table 1. FAB-MS: 365 ($[M+1]^+$), 371 ($[M+Li]^+$), 387 ($[M+Na]^+$). Anal. calc. for $C_{20}H_{28}O_6$: C 65.9, H 7.7; found: C 65.8, H 7.5.

Shikokianin (**3**). White crystalline needles from acetone. M.p. 284–285° (dec.). IR: 3328, 2949, 1728, 1642, 1469, 1369, 1258, 1051, 959. ^{13}C-NMR: *Table 1*.

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REFERENCES

[1] T. Fujita, Y. Takeda, H. D. Sun, Y. Minami, T. Marunaka, S. Takeda, Y. Yamada, T. Togo, *Planta Medica* **1988**, 414.
[2] X. J. Meng, Y. Z. Chen, Y. X. Cui, J. L. Cheng, *J. Nat. Prod.* **1988**, *51*, 812.
[3] B. N. Zhou, J. B. Chen, C. Y. Wang, G. Blasko, G. A. Cordell, *Phytochemistry* **1989**, *28*, 3536.
[4] Z. Q. Wang, X. R. Wang, F. M. Xu, H. P. Wang, *Zhongyao Tongbao* **1987**, *12*, 609.
[5] Z. Q. Wang, X. R. Wang, J. G. Dong, *Acta Bot. Sinica* **1984**, *26*, 634.
[6] J. C. Li, C. J. Liu, X. Z. An, *Acta Bot. Yunnanica* **1984**, *6*, 453.
[7] R. P. Zhang, H. J. Zhang, Z. W. Lin, U. L. Zhen, H. D. Sun, *Phytochemistry* **1992**, *31*, 4237.
[8] G. Y. Xu, *Acta Chim. Sinica* **1985**, *43*, 35.
[9] F. M. Xu, H. P. Hu, Z. Q. Wang, *Zhongguo Zhongyao Zazhi* **1996**, *21*, 678.
[10] K. Osawa, H. Yasuda, T. Maruyama, H. Morita, K. Takeya, H. Itokawa, K. Okuda, *Chem. Pharm. Bull.* **1994**, *42*, 922.
[11] H. D. Sun, Z. W. Lin, F. D. Niu, L. Z. Lin, H. B. Chai, J. M. Pezzuto, G. A. Cordell, *Phytochemistry* **1995**, *38*, 437.
[12] G. Y. Li, W. Z. Song, Q. Y. Ji, Y. L. Wang, *Zhongyao Tongbao* **1984**, *9*, 221.
[13] S. P. S. Bhandari, H. S. Garg, P. K. Agrawal, D. S. Bhakuni, *Phytochemistry* **1990**, *29*, 3956.
[14] IUPAC, *Pure Appl. Chem.* **1999**, *71*, 587.

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