Bitter Principles of *Isodon umbrosus* HARA. The Structures of Umbrosin A and B

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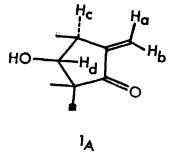
Two new *ent*-kaurene derivatives have been isolated from *Isodon umbrosus* HARA and formulated by spectroscopic and chemical evidence as structures (**1**) and (**2**), respectively. Antimicrobial activity has been tested.

Our continuing investigation of the bitter principles of various *Isodon* species (Labiatae) has led to the isolation of several new diterpenes, which have been assumed to possess enmein or kaurene type skeletons on the basis of spectral and chemical evidence.[1]

We should now like to report the structures of umbrosin A (**1**), $C_{20}H_{30}O_4$, mp 225—228 °C(decomp.), $[\alpha]_D$ −126° (c=1.0, pyridine), and umbrosin B (**2**), $C_{20}H_{28}O_4$, mp 262—265 °C (decomp.), $[\alpha]_D$ −150° (c= 1.0, pyridine). Both compounds display a highly specific antibacterial activity against gram positive bacteria.[2]

Results and Discussion

The main bitter principle, umbrosin A (**1**) exhibits a UV absorption at 233 nm (ε 8400), IR bands at 1730 and 1640 cm^{-1}, and NMR signals at 5.25 and 6.13 δ (1H, broad singlets), typical for a five-membered ring ketone conjugated with an exocyclic methylene group, as exemplified by the D-ring of enmein[3] and oridonin.[4] Its environment was confirmed by spin decoupling, thus: the irradiation of the H_c proton at 3.05 δ (1H), collapsed the broad singlets of the H_a and H_b protons to sharp singlets, showing the removal of allylic coupling. The other broad singlet of the H_d proton at 4.90 δ (1H) also collapsed to a sharp singlet. The appearance of a broad singlet for the H_d proton indicates the dihedral angle between the protons H_c and H_d to be roughly 90°.



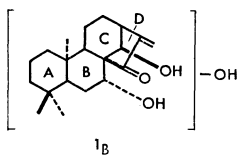
1_A

In addition, the IR spectrum shows the presence of hydroxyl groups at 3300 cm^{-1}, and the NMR spectrum exhibits evidence for three tertiary methyl groups at 0.85, 0.90, and 1.04 δ (3H, singlets), and for two other methine protons on oxygenated carbons at 3.96 and 4.45 δ (discussed later).

Umbrosin A forms a triacetate (**3**), $C_{26}H_{36}O_7$, mp 187—189 °C, of which IR spectrum shows no hydroxyl group, indicating that three oxygen atoms in umbrosin A are unhindered hydroxyl groups. These data and the analogy with congeners isolated from *Isodon* species support a tetracyclic diterpene structure with the *ent*-kaurene type skeleton for umbrosin A, in which the partial structure $\mathbf{1}_A$ would form the D-ring and the hydroxyl group should be situated at C_{14} and α-oriented.

Catalytic hydrogenation of umbrosin A, followed by oxidation with Jones' reagent, gave a diketone (**4**), $C_{20}H_{30}O_4$, of which IR spectrum still shows the presence of hydroxyl groups at 3300 cm^{-1} and a newly produced band due to a six-membered ring ketone at 1710 cm^{-1}. The latter compound yielded a diacetate (**5**), $C_{24}H_{34}O_6$ which exhibits in the NMR spectrum the signals of two acetoxyl groups, indicating that one hydroxyl group was oxidised and the other two were resistant to Jones' oxidative condition. This can be rationalized by assuming an intramolecular hydrogen bond between the two hydroxyl groups, which suggests, in turn, the O—O distance in the –O–H···O–H group is within 3 Å.[5] This assumption is also supported by the formation of an acetonide (**6**), $C_{23}H_{34}O_4$ and a phenylboronate (**7**),[6] $C_{26}H_{33}O_4B$ of umbrosin A, respectively.

In the NMR spectrum of **4**, the signals at 4.34 δ (1H, doublet of doublets, $J_{ax\text{-}ax}$=13 and $J_{ax\text{-}eq}$=5 Hz) and 4.93 δ (1H, broad singlet) were assigned to the protons on carbons bearing hydroxyl group. The appearance of a broad singlet for the latter signal shows that the $C_{14\beta}$ proton still remains in **4** and the coupling constant of the former signal is evidence of the axial nature. An examination of Dreiding model indicates that only when a second hydroxyl group is located at C_7 and β-oriented in the assumed skeleton are the above data explicable. The structure of umbrosin A can therefore be represented by $\mathbf{1}_B$, in which the position and configuration of the third secondary hydroxyl group remain to be settled.



1_B

The mass spectrum of umbrosin A allows the possibility of the existence of the hydroxyl group on the A-ring. Thus, it shows a molecular ion at m/e 334 and intense fragment ions at m/e 316, 301, 298, 283, 194, 176, 140, 122, and 107. The most prominent peaks belong to the fragments formed by cleavage of the B-ring; this can be initiated by α-cleavage of the C_{15} ketonic carbonyl group producing the m/e 194 radical ion **a**. The formation of the ion **a** affords a

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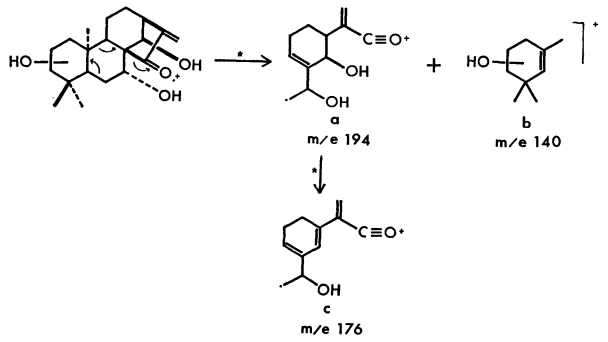
TABLE 1. NMR SPECTRA OF UMBROSIN A AND B, AND SOME DERIVATIVES (in ppm)[a)]

Compounds	CH_3–C	COC=CH_2	$C_{2\alpha}H$–OH	$C_{7\beta}H$–OH	$C_{14\alpha}H$–OH
1	0.85(s) 0.90(s) 1.04(s)	5.25(bs) 6.13(bs)	3.97(tt, $J_{ax-ax}=12$, $J_{ax-eq}=4$)	4.45(dd, $J_{ax-eq}=13$, $J_{ax-eq}=5$)	4.90(bs)
2	0.85(s) 1.05(s) 1.20(s)	5.45(bs) 6.30(bs)		4.65(dd, $J_{ax-ax}=14$, $J_{ax-eq}=5$)	4.95(bs)
3	0.94(s) 0.96(s) 1.30(s)	5.36(bs) 6.12(bs)	5.07(tt, $J_{ax-ax}=12$, $J_{ax-eq}=4$)	5.20(dd, $J_{ax-ax}=14$, $J_{ax-eq}=5$)	6.00(bs)
6	0.87(s) 0.94(s) 1.06(s) 1.20(s) 1.54(s)	5.35(bs) 6.25(bs)	3.83(tt, $J_{ax-ax}=12$, $J_{ax-eq}=4$)	4.16(dd, $J_{ax-ax}=14$, $J_{ax-eq}=5$)	4.53(d, $J=2$)
4	0.86(s) 1.05(s) 1.13(s)			4.34(dd, $J_{ax-ax}=14$, $J_{ax-eq}=5$)	4.93(bs)

a) Determind in C_5D_5N (**1**, **2** and **4**) or in $CDCl_3$ (**3** and **6**) at 100 MHz. Coupling constants are expressed in Hz. s: singlet, bs: broad singlet, dd: doublet of doublets, tt: triplet of triplets.

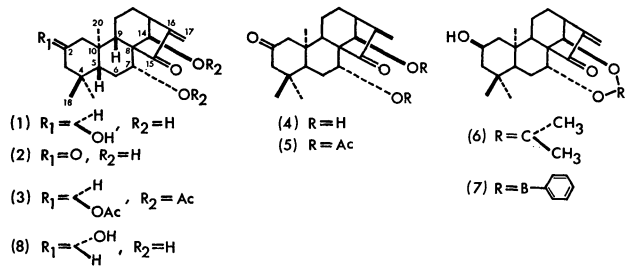
convenient means of locating the remaining hydroxyl group in **1B**.



On the other hand, the NMR spectrum of umbrosin A indicates a signal at 3.97 δ (1H, triplet of triplets, $J_{ax-ax}=12$ and $J_{ax-eq}=4$ Hz) which disappeared on oxidation. The multiplicity and coupling constants of the signal can only arise by interaction of an axial proton with two neibouring axial and two equatorial protons. Hence the third secondary hydroxyl group must be placed at C_2 and α-oriented. The splitting pattern of this proton is quite similar to that of the $C_{2\beta}$ proton of creticoside A isolated from *Pteris cretica* L.[7,8)]

Finally the structure was confirmed by correlation with mebadonin (**8**)[9)] isolated from *Isodon kameba* OKUYAMA, whose structure was determined by X-ray analysis. Catalytic hydrogenation of mebadonin, followed by oxidation with Jones' reagent, gave the same diketone (**4**), and therefore umbrosin A must be represented by **1**, that is *ent*-15-oxokaur-16-ene-2α, 7β,14α-triol. It was also found in *Isodon kameba* OKUYAMA.

A minor bitter principle, umbrosin B (**2**) isolated from a less polar fraction, differs from umbrosin A by having two fewer hydrogens, and shows the presence of a six-membered ring ketone at 1710 cm^{-1} in the IR spectrum. It exhibits the similar NMR to that of umbrosin A, with the exception of lack $C_{2\beta}$ proton at 3.97 δ present in the latter. This suggests that the structure of umbrosin B is *ent*-2,15-dioxokaur-16-ene-7β,14α-diol. Its correlation with umbrosin A was carried out by catalytic hydrogenation of umbrosin B, which yielded **4** in good yield.



Experimental

NMR spectra were determined at 100 MHz using a JEOL PS-100 spectrometer. Mass spectra were measured with a Hitachi RMU-6 spectrometer, high resolution spectra being obtained with a JEOL JMS-D100 instrument. IR spectra were recorded on Nujol mull with a JASCO IRA-1 spectrophotometer. UV data were measured with a Hitachi EPS-2 spectrometer. A Rex Optical Works model NEP-2 was used to measure the optical rotations. Column chromatography was performed on Mallinckrodt silicic acid. Mps were taken in glass capillaries and uncorrected.

Isolation. The dried leaves (1.5 kg) of *Isodon umbrosus* HARA collected near Mt. Amagi (Shizuoka) were extracted with ether (20.5 l). The crude extract was treated with activated charcoal (50 g), concentrated and left to cool overnight. Then a solid (11.4 g) was filtrated off. The filtrate was chromatographed on silicic acid (250 g). Elution with 3% methanol in chloroform gave umbrosin B and A in this order.

Umbrosin A (1). The crude fractions (1.56 g) were recrystallized from ethanol to give pure umbrosin A, as fine needles, mp 225—228° (decomp.), $[\alpha]_D$ −126° ($c=1.0$, pyridine), UV (ethanol) 233 nm (ε 8000), IR (Nujol) 3250, 1730, 1640, 1080 and 1040 cm^{-1}, NMR (see Table 1), Mass *m/e* 334 (M^+), 316 ($M-H_2O$), 298 ($M-2H_2O$), 283 ($M-2H_2O-CH_3$), 194, 176, 149, 122, 107 and 105. (Found: C, 71.34; H, 9.52%. Calcd for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04%),

High resolution mass: 334.2168. $C_{20}H_{30}O_4$ requires: 334.2144.

Umbrosin B (2). The less polar fractions (23 mg) were recrystallized from ethanol to give pure umbrosin B, as needles, mp 262—265° (decomp.), $[\alpha]_D$ −150° (c=1.0, pyridine), UV (ethanol) 232.5 nm (ε 8700), IR (Nujol) 3430, 3200, 1730, 1710, and 1080 cm^{-1}, NMR (see Table 1), Mass m/e 332 (M^+), 314 ($M-H_2O$), 194 and 176. (Found: C, 72.16; H, 8.80%. Calcd for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49%).

Triacetylumbrosin A (3). A solution of umbrosin A (82 mg) in acetic anhydride (1 ml) and pyridine (0.5 ml) was treated as usual manner and recrystallized from acetone–hexane to give the triacetylumbrosin A (**3**), (74 mg), as fine needles, mp 187—189°, IR (Nujol) 1720, 1640 and 1230 cm^{-1}, NMR (see Table 1) also 1.93, 2.00, and 2.05 δ (3H, singlets, $OCOCH_3$), (Found: C, 67.62; H, 8.35%. Calcd for $C_{26}H_{36}O_7$: C, 67.80; H, 7.88%).

Bisdehydrodihydroumbrosin A (4). Umbrosin A (190 mg) in ethanol (10 ml) was hydrogenated over 10% Pd-C (20 mg) for 3 hr. The catalyst was removed by filtration. Then the filtrate was evaporated and crystallized from ethanol to yield the dihydroumbrosin A (185 mg), as rods, mp 239—243 °C (decomp.), IR (Nujol) 3300, 3080, and 1740 cm^{-1}, (Found: C, 71.05; H, 9.43%. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59%). To a solution of dihydroumbrosin A (100 mg) in acetone (25 ml) was added Jones' reagent (1 ml) dropwise with stirring and ice cooling. The reaction was complete within 10 min, the mixture was stirred for further 10 min, then worked up by dilution with water (25 ml) and concentrated *in vacuo* to remove acetone. The white precipitate was filtered off and chromatographed on silicic acid (1 g). Elution with chloroform gave the bisdehydrodihydroumbrosin A (83 mg) which was recrystallized from ethanol to give the pure compound, as needles, mp 254—259° (decomp.), $[\alpha]_D$ −126° (c=1.0, pyridine) IR (nujol) 3450, 3200, 1740, and 1710 cm^{-1}, NMR (see Table 1), strongly positive Zimmermann test, (Found: C, 71.95; H, 9.13%. Calcd for $C_{20}H_{30}O_4$: 71.82; H, 9.04%).

Acetylbisdehydrodihydroumbrosin A (5). Bisdehydrodihydroumbrosin A (45 mg) was treated as described above to yield the acetylbisdehydrodihydroumbrosin A (37 mg), as needles, mp 178—179 °C, IR (Nujol) 1740, 1710, and 1260 cm^{-1}, NMR ($CDCl_3$) 1.90 and 2.00 δ (3H, singlets, $OCOCH_3$).

Isopropylideneumbrosin A (6). To a solution of umbrosin A (150 mg) in acetone (25 ml) was added concd sulfuric acid (2 drops). The mixture was left at room temperature for 6 days and then extracted with chloroform (200 ml). The extract was washed with sodium bicarbonate solution and water, dried over magnesium sulfate and evaporated to dryness. The residue was chromatographed on silicic acid, elution with chloroform gave the isopropylideneumbrosin A (51 mg), which was recrystallized from acetone–hexane to yield the pure material, as needles, mp 200—201 °C, IR (Nujol) 3320, 1730, 1645, 1115, and 1030 cm^{-1}, NMR (see Table 1), (Found: C, 73.65; H, 9.35%. Calcd for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64%).

Umbrosin A-phenylboronate (7). Umbrosin A (105 mg) was treated in boiling benzene (25 ml) with phenylboronic acid (55 mg); water was removed with molecular sieves. Removal of the main part of the benzene and addition of ethanol caused the crystallization of the product; almost quantitative yield was obtained. Recrystallization from ethanol gave the umbrosin A phenylboronate (134 mg), as plates, mp 218—219 °C, IR (Nujol) 3550, 1730, 1640, 1600, and 1300 cm^{-1}, Mass m/e 420 (M^+).

Bisdehydrodihydromebadonin (4). Mebadonin (88 mg) was treated as described above to give the bisdehydrodihydromebadonin (**4**) which was identified by mixed mp., IR, and NMR comparison with the bisdehydrodihydroumbrosin A.

Dihydroumbrosin B (4). Umbrosin B (85 mg) was hydrogenated as described above to give the dihydroumbrosin B (**4**), which was identified by mixed mp., IR, and NMR comparison with the bisdehydrodihydroumbrosin A.

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