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# THE STRUCTURES OF DITERPENE GLYCOSIDES, SHIKOKIASIDE A AND B

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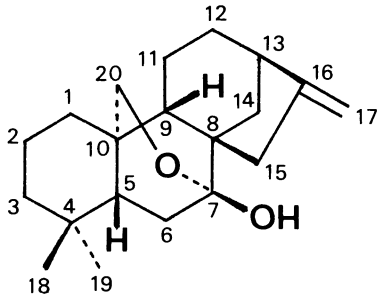
The structures of two new diterpene glycosides, shikokiaside A and B, isolated from Rabdosia shikokiana var. shikokiana were elucidated, respectively, by chemical and spectroscopic data.

From the Rabdosia species[1] (Labiatae) about sixty diterpenes having ent-kaurene or its seco type had been isolated and their structures were elucidated. The diterpenoids which were previously isolated from R. shikokiana (Makino) Hara var. shikokiana were shikokianin (1)[2], oridonin[2], shikokianidin[3], shikodonin[4], and other four ent-kaurenoid compounds[5]. Further investigation of the same plant has led to the isolation of two new diterpene glycosides. The isolation of these compounds are the first case from Rabdosia species.

The dry leaves (6 kg) were extracted with $Et_2O$ and further with MeOH. The extract was concentrated and divided into the soluble parts of $Et_2O$, EtOAc, n-BuOH, and $H_2O$. The droplet counter current chromatography ($CHCl_3$-MeOH-$H_2O$-n-BuOH, 10 : 10 : 6 : 1) of the concentrate of the n-BuOH-soluble fraction gave shikokiaside A and B (each about 200 mg).

Shikokiaside A (1), mp 235-240°C, $[\alpha]_D^{25}$ +0.5° (c=0.8, $C_5H_5N$), Found: C, 55.35; H, 7.72%. Calcd for $C_{26}H_{40}O_{11}\cdot 2H_2O$: C, 55.31; H, 7.85%, showed the presence of a hydroxyl group (IR[6], $\nu_{max}$ 3400, 1070, 1040 $cm^{-1}$) and an exocyclic methylene group ($^{13}C$ NMR, δ 162.8, 106.6). Shikokiaside B (2), mp 265-268°C, $[\alpha]_D^{25}$ -15.3° (c=1.2, $C_5H_5N$), Found: C, 58.36; H, 8.01%. Calcd for $C_{26}H_{40}O_{10}\cdot H_2O\cdot\frac{1}{2}C_2H_5OH$: C, 58.57; H, 8.19%, had also a hydroxyl group ($\nu_{max}$ 3350, 1070, 1040 $cm^{-1}$) and an exocyclic methylene group (δ 162.6, 106.7). There was no absorption in the UV spectra of 1 and 2. From FDMS spectra the molecular ion peaks of 1 and 2 were observed as m/e 528 and 512, respectively. There is one glycosyl group in each compound having the molecular weight of 180 in 1 and 2 because the fragment ion peaks of 1 and 2 show m/e 366 and 350 from EIMS spectra (70 eV).

The hydrolysis of 1 and 2 with the crude hesperidinase[7] gave aglycones 3, mp 259-261°C and 4, mp 251-254°C, respectively, which showed the presence of the hydroxyl groups by IR spectra. The hydrolytic reaction of 1 was found to be slow in comparison with 2. There are three secondary hydroxyl groups in 3 [ EIMS, m/e 366 ($M^+$, $C_{20}H_{30}O_6$), 348 ($M^+-H_2O$), 330 ($M^+-2H_2O$), 312 ($M^+-3H_2O$); $^1H$ NMR[8],



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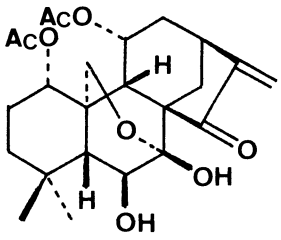

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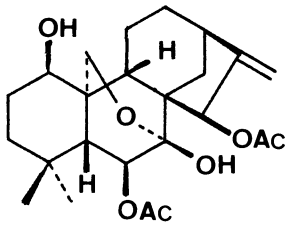

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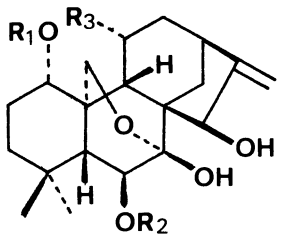
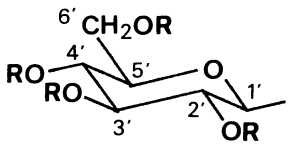


| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 1 | Glc | H | OH |
| 2 | Glc | H | H |
| 3 | H | H | OH |
| 4 | H | H | H |
| 5 | 4Ac-Glc | Ac | OH |
| 6 | 4Ac-Glc | H | OH |
| 7 | 4Ac-Glc | Ac | H |
| 8 | 4Ac-Glc | H | H |
| 9 | Ac | Ac | H |
| 10 | Ac | H | H |



Glc R = H

4Ac-Glc R = Ac

δ 3.87 (t, J=8 Hz), 4.19 (d, J=6 Hz), 4.54 (t, J=4 Hz)] while two hydroxyl groups in 4 [ m/e 350 ($M^+$, $C_{20}H_{30}O_5$), 332 ($M^+-H_2O$), 314 ($M^+-2H_2O$); δ 3.67 (t, J=8 Hz), 4.13 (d, J=6 Hz)] at least. It was presumed that 3 and 4 had the skeleton, ent-7β,20-epoxy-kaur-16-en-7α-ol (11) and the positions of the hydroxyl groups were C-1, C-6, C-15, and C-11 in 3 still under investigation by $^1H$ NMR and $^{13}C$ NMR spectral data. Compounds 3 and 4 were identical with the authentic samples derived from shikokianin (12)[2,3] and from trichokaurin (13)[9], respectively, by IR, MS, and TLC.

The signals of $^{13}C$ NMR spectra were assigned according to the values reported by Ochi et al[4] (Table 1). The values of chemical shifts of 1 and 2 were similar except for the values of C-5, C-9, C-11, C-12, and C-20. The upfield shifts of the β-carbon by the glycosidation[10] were 1.1 ppm (C-2), 1.5 (C-5), and 1.2 (C-11) in 1, and 2.4 (C-2) in 2. These results indicate that a sugar may be linked at C-1 or C-6 in 1 and C-1 in 2.

The acetylation of 1 and 2 with $Ac_2O$-$C_5H_5N$ gave a pentaacetate together with a tetraacetate, respectively; 5, mp 196-199°C and 6, mp 239-241°C from 1, and 7, mp 252-254°C and 8, mp 279-281°C from 2. On addition of a catalytic amount of 4-dimethylaminopyridine 1 and 2 gave only the pentaacetates (5 and 7). The tetraacetates are derived from the acetylation of the sugar moiety, and the additional acetate in the pentaacetates is due to further acetylation of one of the hydroxyl groups of

Table 1. $^{13}C$ Chemical shifts (δ) of compounds 1, 2, 3, 4, 5, 7, and 9. [a]

| Carbon no. | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
|---|---|---|---|---|---|---|---|
| C-1 | 85.1 | 84.5 | 74.0 | 73.9 | 85.4 | 84.2 | 76.9* |
| C-2 | 28.5 | 28.4 | 29.6 | 30.8 | 26.5 | 27.5 | 25.9 |
| C-3 | 39.3 | 39.2 | 39.3 | 39.5 | 38.4 | 38.2 | 37.9 |
| C-4 | 33.5 | 33.6 | 33.9 | 34.1 | 32.6 | 33.3 | 33.6 |
| C-5 | 56.9 | 58.7 | 58.4 | 58.3 | 54.2 | 54.8 | 54.9 |
| C-6 | 75.3* | 74.9* | 75.3* | 75.0* | b | b | 76.1* |
| C-7 | 97.3 | 97.0 | 97.8 | 97.3 | 95.8 | 95.3 | 95.8 |
| C-8 | 52.6 | 52.8 | 52.4 | 52.8 | 51.0 | 51.5 | 51.5 |
| C-9 | 48.6 | 43.6 | 47.8 | 43.9 | 47.1 | 43.0 | 42.6 |
| C-10 | 42.7 | 41.6 | 42.5 | 41.5 | 42.2 | 41.1 | 39.8 |
| C-11 | 65.4 | 19.3 | 66.6 | 19.2 | 65.4 | 18.3 | 16.8 |
| C-12 | 45.3 | 33.2 | 42.9 | 33.3 | 42.6 | 32.5 | 32.1 |
| C-13 | 36.9 | 37.3 | 37.5 | 37.2 | 35.5 | 35.8 | 35.9 |
| C-14 | 26.7 | 26.9 | 26.9 | 26.9 | 25.4 | 25.7 | 25.1 |
| C-15 | 76.0* | 75.7* | 75.8* | 75.4* | 74.7 | 74.1 | 74.0 |
| C-16 | 162.8 | 162.6 | 162.6 | 162.7 | 159.8 | 159.7 | 159.2 |
| C-17 | 106.6 | 106.7 | 106.3 | 106.9 | 108.1 | 108.4 | 108.3 |
| C-18 | 22.5 | 22.1 | 22.4 | 22.3 | 22.5 | 22.0 | 22.0 |
| C-19 | 34.1 | 33.2 | 33.7 | 33.3 | 33.3 | 32.1 | 31.8 |
| C-20 | 65.9 | 63.7 | 65.8 | 63.6 | 65.4 | 63.5 | 63.5 |
| C-1′ | 104.8 | 104.9 | | | 99.7 | 100.5 | |
| C-2′ | 75.3 | 75.3 | | | 71.8 | 71.7 | |
| C-3′ | 78.9 | 79.0 | | | 73.5 | 73.4 | |
| C-4′ | 71.6 | 71.7 | | | 68.3 | 68.7 | |
| C-5′ | 78.6 | 78.3 | | | 71.8 | 71.7 | |
| C-6′ | 62.7 | 62.9 | | | 62.3 | 62.4 | |

a) Compounds 1, 2, 3, and 4 were measured in $C_5D_5N$ and the others were measured in $CDCl_3$, using tetramethylsilane as internal standard. b) These signals were not observed by the influence of the signal of solvent. c) The chemical shifts of acetyl groups were δ 170.6, 170.4, 169.4, 21.7 21.0, 20.8, and 20.7 in 5, 170.8, 170.6, 170.4, 169.5, 169.1, 21.7, 20.9, and 20.8 in 7, and 171.0, 170.0, and 21.7 in 9. d) Assignments at C-6 and C-15 in 1, 2, 3, and 4, and at C-1 and C-6 in 9 are mutually exchangeable.

the aglycones. The sugar part was decided as β-D-glucoside from $^{13}C$ NMR spectra of glycosides 1 and 2, and their acetates 5 and 7[10], respectively. The magnitude of the coupling constant of the signal of δ 4.72 (d, J=10 Hz) in 2 appears reasonable for β-glycosidic linkage. The signals of δ 5.30 (d, J=8 Hz) and 5.19 (d, J=8 Hz) are attributed to the protons of C-6 in 5 and 7, respectively, shifted to downfield comparing with those of 3 and 4. The signals of δ 54.2 and 54.8 in the $^{13}C$ NMR spectra of 5 and 7, respectively, are similar to those of δ 54.9 of diacetate 9, mp 207-209°C, which together with a small amount of the by-product, monoacetate 10, mp 264-268°C, was derived from the acetylation ($Ac_2O$-$C_5H_5N$) of 4. These results indicate that the fifth acetoxyl group in 5 and 7 is attached to C-6, and glucose may be linked at C-1. If the glucose would attach to C-15, the hydroxyl group at C-6 will not react under acetylating condition with the steric hindrance. The difference between the rates of the enzymic hydrolysis in 1 and 2 is explicable as the influence by the presence of the hydroxyl group at C-11 in 1.

From these experimental results, the structures of shikokiaside A and B were determined as ent-7β,20-epoxykaur-16-ene-1β,6α,7α,11β,15α-pentaol 1-O-β-D-glucopyranoside (1) and ent-7β,20-epoxykaur-16-ene-1β,6α,7α,15α-tetraol 1-O-β-D-glucopyranoside (2), respectively.

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