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A DAMMARANE SAPONIN FROM *NEOALSOMITRA INTEGRIFOLIOLA*

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Key Word Index—*Neoalsomitra integrifoliola*; Cucurbitaceae; dammarane saponin; ocotillone-type triterpene; neoalsoside A; neoalsogenin A.

Abstract—Neoalsoside A, a new dammarane saponin, was isolated from *Neoalsomitra integrifoliola* and characterized as 12β,23β,25-trihydroxy-(20*S*)(24*S*)-epoxydammarane-3-*O*-α-L-rhamnosyl(1→2)-α-L-rhamnosyl(1→3)-β-D-glucoside.

INTRODUCTION

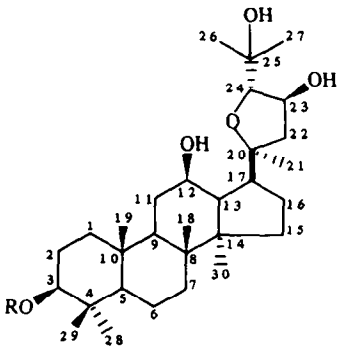
Neoalsomitra integrifoliola (Cong.) Hutch is a herb growing in southern China as well as in Southeast Asia. From rhizomes of this plant, we have isolated a major dammarane saponin in a high yield (over 3%). The present communication describes the structural elucidation of this saponin named neoalsoside A and its aglycone, neoalsogenin A. This study shows that *N. integrifoliola* is another plant source of ginseng-saponin.

RESULTS AND DISCUSSION

An ethanolic extract of dried rhizomes was separated as described in the Experimental, affording a new dammarane saponin neoalsoside A (**1**) in a high yield (over 3%). On acidic hydrolysis, saponin **1** gave glucose (Glc) and rhamnose (Rha) as sugar components (by TLC) and a new aglycone neoalsogenin A (**2**). Aglycone **2**, needles (acetone), mp 215–218°, $[\alpha]_D$ +11.2° (pyridine), showed an $[M+H]^+$ ion at *m/z* 493 in the FAB mass spectrum, and exhibited 30 carbons (Me- × 8, $-CH_2-$ × 8, $-\overset{|}{C}H$ × 4, $-O-\overset{|}{C}H$ × 4, $-O-\overset{|}{C}-$ × 2, $-\overset{|}{C}-$ × 4) in ^{13}C NMR, indicating a molecular formula of $C_{30}H_{52}O_5$. The characteristics of ^{13}C NMR data of **2** strongly suggest that it is an ocotillone-type triterpene [1–3], and the ^{13}C NMR signals of skeleton were easily assigned as in Table 1. The presence of 3β and 12β hydroxyl groups in **2** deduced from ^{13}C NMR signals at δ78.9 and 70.6, respectively, were further confirmed by ^1H NMR signals at δ3.19 (*dd*, *J* = 12.0, 5.0 Hz, H-3) and 3.54 (*ddd*, *J* = 12.0, 12.0, 5.0 Hz, H-12), respectively. The CH_2 signal at 18.3 indicated the absence of oxygenation at C-6. The signal at 89.7 (C-24) suggested the presence of 20,24-epoxy ring and 24*S* configuration [1]. In the FAB mass spectrum of **2**, the characteristic base peak at *m/z* 143 corresponding to the side chain containing a 20,24-epoxy group in ocotillone-type triterpene was not observed [1], but instead of this peak an intense peak was noticed at *m/z* 159, suggesting the presence of a hydroxyl group in the side chain. The presence and β-orientation of the hydroxyl group at C-23 was estimated by the coupling constant (*J* = 8.1 Hz) between H-23 and H-24 and by those (*J* = 8.0, 0.0 Hz) between H-23 and H_2-22. The stereochemistry of H-23α and H-24β was confirmed by observing NOEs between H-23 and the protons of Me-21, Me-26 and Me-27. These NOEs agreed with the previous estimation about the configuration at C-24.

On the other hand, a strong support for the side chain structure was provided by the FAB mass spectrum of triacetate (**3**) of **2**, in which two intense peaks at *m/z* 141 (base peak) and 201 (58%) are reasonably ascribed to the fragment ions, $[C_{10}H_{17}O_4-HOAc]^+$ and $[C_{10}H_{17}O_4]^+$, respectively, both being derived from the side chain. Thus, **2** is 3,12,23,25-tetrahydroxy-(20*S*),(24*S*)-epoxydammarane, neoalsogenin A.

Saponin **1**, needles (acetone), mp 277–279°, $[\alpha]_D$ −30.5° (pyridine), has the molecular formula $C_{48}H_{82}O_{18}$ by FAB mass spectroscopy (*m/z* 969, $[M+Na]^+$) and ^{13}C NMR (DEPT). The attachment of the sugar chain at the C-3 position of **2** was evident by the downfield shift (9.91 ppm) of C-3 in **2** compared with C-3 in **1** [3, 4]. The ^{13}C and ^1H NMR spectra indicated the presence of two



R

1 Glc(2→Rha, 3→Rha)

2 H

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Table 1. ^{13}C NMR chemical shifts

C	1 ($CDCl_3$)	2 (C_5D_5N)	C	1 ($CDCl_3$)	2 (C_5D_5N)	Sugar moiety of 1 (C_5D_5N)	
1	39.8	39.0	21	27.8	26.5	Glc-1	105.1
2	27.0	27.4	22	42.3	40.5	2	78.1[a]
3	88.8	78.9	23	71.0	70.1	3	87.5
4	40.1	39.0	24	91.7	89.7	4	70.7[b]
5	56.8	56.0	25	70.4	70.8	5	78.1[a]
6	18.5	18.3	26	26.7	25.7	6	62.7
7	35.3	34.8	27	29.9	29.7	2-Rha-1	102.3
8	39.8	39.8	28	28.0	28.0	2	72.1[c]
9	50.7	50.3	29	16.9	15.4	3	72.6[c]
10	37.2	37.2	30	18.3	17.9	4	72.7[c]
11	32.7	31.6				5	70.3[b]
12	70.9	70.6				6	18.6
13	49.1	49.0				3-Rha-1	103.8
14	52.5	52.2				2	72.7[c]
15	32.6	32.2				3	72.6[c]
16	28.7	28.5				4	73.9
17	50.1	49.3				5	70.5[b]
18	16.8	16.3				6	18.7
19	15.7	15.6					
20	85.3	85.5					

[a–c]Signals thus indicated may be reversed.

terminal α-L-rhamnopyranosyl units and an inner β-D-glucopyranosyl unit (anomeric carbons: δ102.3, 103.8 and 105.1; anomeric protons: δ5.68 (*br s*), 5.45 (*br s*) and 4.58 (*d*, $J = 7.8$ Hz) and characteristic carbons: δ18.6 (Me), 18.7 (Me) and 62.7 (CH_2). According to the chemical shifts [5], the ^{13}C NMR signals of inner β-glucosyl unit at δ82.6 and 78.1 suggested that the sugar moiety is 2,3-di-*O*-α-L-rhamnosyl-β-*O*-glucopyranoside. All sugar carbon signals were identical to those of the sugar moieties of taccaoside [6]. Accordingly, the structure of **1** is 12β,23β,25-trihydroxy-(20*S*),(24*S*)-epoxydammarane 3-*O*-α-L-rhamnopyranosyl(1→2) [α-L-rhamnopyranosyl (1→3)]-β-D-glucopyranoside, neoalsoside A.

EXPERIMENTAL

Plant material. Rhizomes of *N. integrifoliola* (Cogn.) Hutch were collected in Xishuangbanna, South-Yunnan, China, and identified by Prof. Tao Guoda. A specimen is deposited in the Herbarium of the Kunming Institute of Botany.

Isolation of neoalsoside A (**1**). Air-dried powdered materials (450 g) were extracted with 95% EtOH under reflux. The extract was evapd to afford a syrup (40 g), which was dissolved in MeOH. After removing the insoluble materials by filtration, the filtrate was concd *in vacuo* to give a residue (32.4 g). The residue was subjected to CC on silica gel (75 g), which was eluted stepwise with $CHCl_3$–MeOH solvent of increasing polarity. Concn of the 20% MeOH eluate gave a crude neoalsoside A, which was then passed through a reverse-phase column (RP-18, 40 g) to afford pure neoalsoside A (**1**) (*ca* 14 g).

Neoalsoside A (**1**). $C_{48}H_{82}O_{18}$, needles (acetone), mp 225–228°, $[\alpha]_D$ −30.5° (pyridine). FAB-MS *m/z* (%): 969 ($[M+Na]^+$, 30), 457 (80), 391 (25), 307 (70), 120 (base peak, 100). ^1H NMR: δ (pyridine-d_5) 5.81 (1H, *s*, OH-12), 5.68 (1H, *br s*, Rha-1″), 5.45 (1H, *br s*, Rha-1′), 4.58 (1H, *d*, $J = 7.8$ Hz, Glc-1), 3.74 (1H, *d*, $J = 8.1$ Hz, H-24), 1.39, 1.35 (each 3H, *d*, $J = 6.5$ Hz, 2 × Rha-Me), 1.34, 1.32, 1.15 (each 3H, *s*, Me-21, Me-26, Me-27). ^{13}C NMR and DEPT: as shown in Table 1. ^1H–^{13}C COSY spectrum was used in the assignment of ^1H and ^{13}C NMR signals.

Acidic hydrolysis of **1**. A solution of **1** (100 mg) in 2 M HCl–50% dioxane (30 ml) was refluxed for 4 hr. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and dried over Na_2SO_4. Then, the $CHCl_3$ solution was filtered and the filtrate was evapd to dryness. The residue was passed through a RP-18 column to afford aglycone (**2**). Neoalsogenin (**2**), needles (acetone), mp 215–218, $[\alpha]_D$ +11.2 (pyridine). FAB-MS *m/z* (%): 493 ($[M+H]^+$, 30), 391 (80), 159 (base peak, 100). ^1H NMR δ($CDCl_3$): 5.55 (1H, *s*, OH-12), 4.55 (1H, *br dd*, $J = 8.1$, 8.0 Hz, H-23), 3.62 (1H, *d*, $J = 8.1$ Hz, H-24), 3.54 (1H, *ddd*, $J = 12.0$, 12.0, 5.0 Hz, H-12), 3.19 (1H, *dd*, $J = 12.0$, 5.0 Hz, H-3), 1.29, 1.26, 1.24 (each 3H, *s*, Me-21, Me-27, Me-26), 1.04, 0.98, 0.91, 0.90, 0.78 (each 3H, *s*, Me-28, Me-29, Me-30, Me-19, Me-18). ^{13}C NMR data: as shown in Table 1. ^1H–^1H COSY and ^1H–^{13}C COSY spectra were used in the ^1H and ^{13}C NMR signal assignments.

3,12,23-*Triacetate* (**3**). FAB-MS *m/z* (%): 619 ($[M+H]^+$, 10), 559 ($[M+H-HOAc]^+$, 25), 541 ($[M+H-HOAc-H_2O]$, 40), 499 ($[M+H-HOAc\times2]^+$, 20), 481 ($[M+H-HOAc\times2-H_2O]$, 45), 201 ([side chain $(C_{10}H_{17}O_4)$]$^+$, 58), 141 ($[C_{10}H_{17}O_4-HOAc]^+$, base peak).

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REFERENCES

1. Tanaka, O. and Yahara, S. (1978) *Phytochemistry* **17**, 1353.
2. Tanaka, O., Morita, T., Kasai, R., Kinouchi, J., Sanada, S., Ida, Y. and Shoji, J. (1985) *Chem. Pharm. Bull.* **33**, 2323.
3. Tanaka, O. (1985) *Yakugaku Zasshi* **105**, 323.
4. Seo, O., Tomita, Y., Tori, K. and Yoshimura, Y. (1978) *J. Am. Chem. Soc.* **100**, 3331.
5. Agrawal, P. K., Jain, D. C., Gupta, P. K. and Thakur, R. S. (1985) *Phytochemistry* **24**, 2479.
6. Zhou, J., Chen, C.-X., Liu, R.-M. and Yang, C.-R. (1983) *Acta Botanica Sinica* **25**, 568.