Pergamon

0031-9422(94)00846-9

Phytochemistry, Vol. 39, No. 1, pp. 99–103, 1995

0031-9422/95 $9.50 + 0.00

TERPENOIDS FROM THE LIVERWORTS *SYMPHYOGYNA BRASILIENSIS* AND UNIDENTIFIED *FRULLANIA* SPECIES

MOTOO TORI, MAMIKO AOKI, KATSUYUKI NAKASHIMA and YOSHINORI ASAKAWA

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro cho, Tokushima 770, Japan

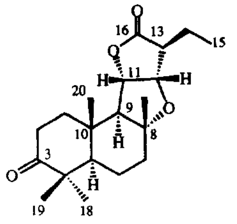
(*Received* 31 *August* 1994)

Key Word Index—*Symphyogyna brasiliensis*; Pallaviciniaceae; *Frullania* sp.; Frullaniaceae; labdane-type diterpene; symphyogynolide; rothin-A acetate; α-cyclocostunolide; methyl 3α-hydroxyolean-18-en-28-oate; sesquiterpene; triterpene; liverwort; Hepaticae.

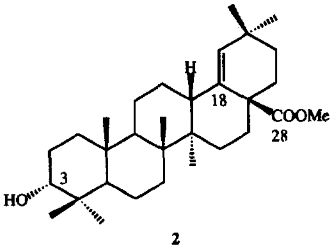
Abstract—Symphyogynolide, a new labdane-type diterpene, has been isolated from the Venezuelan liverwort *Symphyogyna brasiliensis* Nees. A new Δ^{18} oleanane-type triterpene ester, as well as the previously known rothin-A acetate, α-cyclocostunolide and a farnesane-type lactone, have been isolated from some unidentified Venezuelan liverworts (*Frullania* species) and their structures determined by spectroscopic techniques.

INTRODUCTION

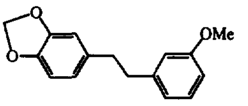
We have recently reported on the isolation and characterization of some interesting chemical constituents from species of the liverwort genera *Frullanoides* [1, 2], *Porella* [3, 4] and *Marchantia* [5] collected in South America. There are many species of liverwort in South America and they are interesting from the chemical point of view [6–8]. *Pallavicinia* species have been extensively studied and many sesquiterpenoids have been isolated [9–13]. However, most compounds so far isolated are limited to sesquiterpenoids [9–12]. We have investigated the chemical constituents of the liverwort *Symphyogyna brasiliensis*, which belongs to the subfamily Pallavicinia, and found a diterpenoid, named symphyogynolide (**1**), which is the second example of a diterpenoid from this family. Two unidentified *Frullania* species yielded a new Δ^{18} oleanane-type triterpene ester (**2**) and the previously known bibenzyl (**3**) [14, 15], α-cyclocostunolide (**4**) [16], rothin-A acetate (**5**) [17], hinesene (**6**) [18], and *ent*-cyclocolorenone (**7**) [16]. A farnesane-type lactone was isolated as a natural product for the first time, although it was known previously as a synthetic intermediate [19–22].



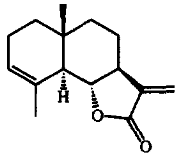
1

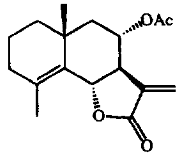


2

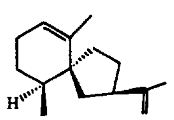


3

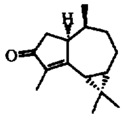

4

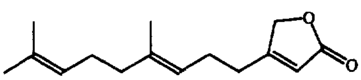


5



6


7


8

RESULTS AND DISCUSSION

The ether extract of *S. brasiliensis* was purified by a combination of Sephadex LH-20 and silica gel CC as well as preparative TLC to afford compound **1**; it exhibited a molecular ion peak at *m/z* 334 and the molecular formula $C_{20}H_{30}O_4$ was determined by HRMS. Since the ^{13}C NMR spectrum showed 20 peaks, it had to be a diterpene. Five methyls, one of which showed a triplet in the ^{1}H NMR spectrum, were rather congested in CDCl$_3$, but they were well separated in C$_6$D$_6$ (Table 1). Thus, the analysis was carried out using C$_6$D$_6$ as solvent. The IR spectrum indicated the presence of a lactone (1780 cm^{-1}) and a carbonyl (1700 cm^{-1}). The 2D NMR spectra suggested the partial structures A–C shown in Fig. 1. The HMBC spectrum was used to connect these partial structures to construct a labdane skeleton (Table 2). The configuration was determined by the NOESY spectrum (Fig. 2). From the above data symphyogynolide (**1**) was established as a labdane-type diterpene keto-lactone as depicted in the formula. However, the absolute configuration was not determined.

The ether extract of the unidentified *Frullania* species (No. 3122) was separated by a combination of Sephadex and silica gel CC and HPLC to afford a new triterpene (**2**) as well as the previously known bibenzyl (**3**) [14, 15], (+)-α-cyclocostunolide (**4**) [16], and rothin A acetate (**5**) [17]. The extract of another unidentified *Frullania* species (No. 3123) was similarly purified to afford **4**, **5**, hinesene (**6**) [18], *ent*-cyclocolorenone (**7**) [16] and a new farnesane-type compound (**8**).

Compound **2** exhibited a molecular ion peak at *m/z* 470 in its mass spectrum and its molecular formula was determined as C$_{31}$H$_{50}$O$_3$ by HRMS. The IR spectrum showed absorptions at 3600 (hydroxyl) and 1720 (car-

Table 1. ^{1}H and ^{13}C NMR data for symphyogynolide (**1**)

H/C	400 MHz, CDCl$_3$		600 MHz, C$_6$D$_6$	
1α	1.77 *m*	37.3	1.29 *m*	37.1
1β	1.92 *m*	—	1.54 *ddd* (13.6, 7.8, 3.6)	—
2α	2.60 *m*	33.5	2.22 *ddd* (16.3, 7.8, 3.6)	33.4
2β	2.48 *dd* (16.6, 7.2, 3.4)	—	2.27 *ddd* (16.3, 10.4, 7.8)	—
3	—	215.9	—	213.5
4	—	47.4	—	47.0
5	1.51 *m*	54.8	0.90 *m*	54.0
6a	1.75 *m*	21.5	1.17 *m*	21.3
6b	1.48 *m*	—	0.88 *m*	—
7α	1.56 *m*	38.9	1.20 *m*	39.9
7β	1.98 *m*	—	1.59 *m*	—
8	—	82.3	—	81.9
9	1.55 *d* (9.3)	66.2	1.05 *d* (9.4)	65.7
10	—	35.8	—	35.5
11	4.91 *dd* (9.0, 7.2)	80.0	4.15 *dd* (9.4, 7.3)	79.4
12	4.53 *dd* (7.2, 3.7)	79.8	4.00 *dd* (7.3, 4.0)	79.9
13	2.57 *m*	48.6	2.49 *ddd* (9.0, 4.8, 4.0)	49.1
14a	1.96 *m*	23.3	1.82 *m*	23.5
14b	1.63 *m*	—	1.39 *m*	—
15	1.062 *t* (7.3)	11.4	0.89 *t* (7.3)	11.6
16	—	178.0	—	177.0
17	1.26 *s*	21.9	0.76 *s*	21.7
18	1.13 *s*	26.8	0.99 *s*	26.8
19	1.056 *s*	20.9	0.84 *s*	20.8
20	1.07 *s*	15.1	0.54 *s*	14.9

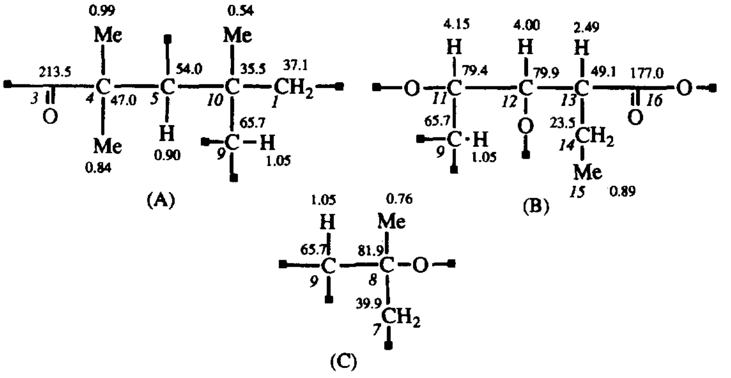


Fig.1. Partial structures of symphyogynolide (**1**).

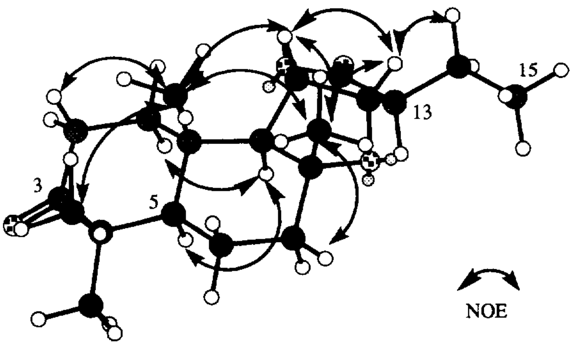


Fig. 2. NOEs detected for symphyogynolide (1).

Table 2. HMBC correlations for symphyogynolide (1)

C	Correlated protons
1	2α, 2β, 20
2	1β
3	1, 2, 18, 19
4	18, 19
5	1β, 7β, 18, 19, 20
7	9, 17
8	6a, 7β, 9, 17
9	7β, 17, 20
10	1β, 6a, 9, 20, 11
11	9, 12
12	9, 14a, 14b
13	14a, 14b, 15
14	12, 15
15	14a, 14b
16	11, 12, 14a, 14b

Table 3. ^{13}C NMR data for **2**

C	δ (ppm)	C	δ (ppm)
1	33.6	16	33.6
2	25.4	17	48.2
3	76.2	18	137.2
4	37.6	19	132.5
5	49.2	20	32.0
6	18.1	21	33.6
7	34.4	22	33.6
8	40.8	23	28.2
9	50.9	24	22.1
10	37.4	25	16.4
11	20.8	26	15.9
12	26.0	27	15.0
13	41.2	28	177.3
14	42.6	29	30.4
15	29.3	30	29.1
		-OMe	51.9

Table 4. HMBC correlations for **2**

H	Correlated carbons
3	1, 2
19	13, 17, 18
23	3, 4, 5, 24
24	3, 4, 5, 23
25	5, 9, 10
26	8, 9, 14
27	8, 13, 14, 15
29	19, 20, 21, 30
30	19, 20, 21, 29

bonyl) cm^{-1}. These functional groups were further supported by the ^{13}C NMR spectrum (δ76.2 and 177.3) (Table 3). Eight methyls, 10 methylenes, five methines and four quaternary carbons were revealed by the DEPT spectra. It contained five carbocycles, because its degree of unsaturation was seven and it had an olefin and carbonyl group. From these results, compound **2** had to be a pentacyclic triterpene methyl ester with one hydroxyl group. The 2D NMR spectra suggested that it was an oleanane-type triterpene with an axial hydroxyl group at C-3 and a methoxycarbonyl group at C-28 as shown by the HMBC correlation (Table 4). The configuration was determined from the NOESY spectrum (Fig. 3).

Compound **8**, $C_{15}H_{22}O_2$, displayed absorptions at 1780 and 1750 cm^{-1} in its IR spectrum. The ^{13}C NMR spectrum exhibited 15 carbon signals and the ^{1}H NMR spectrum showed three olefinic protons and three olefinic methyl groups. From these data structure **8**, a compound previously reported as a synthetic intermediate [19–22], was formulated. Although **8** is a known compound, this is the first report of its isolation from a natural source.

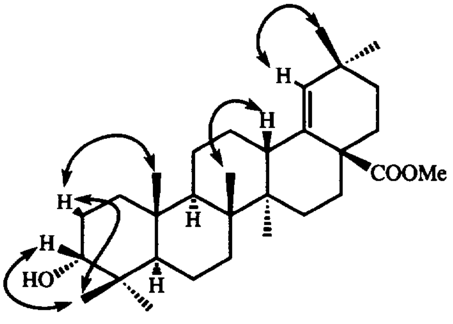


Fig. 3. NOEs detected for **2**.

Symphyogyna has rarely been investigated so far, and a diterpenoid has never been isolated. Wu [13] recently reported a highly rearranged secolabdane lactone, pallavicinin, from *Pallavicinia subciliata* [13], which is similar to compound **1** to some extent. Isolation of compound **1** suggests that these two species are related to each other taxonomically. To our regret, the *Frullania* sp. have not been identified yet. However, our chemosystematics investigations clearly indicate that they must belong to chemotype II (sesquiterpene–bibenzyl) [6]. The oleanane-type triterpene has never been isolated from liverworts before. Since this compound was isolated as one of the major constituents, it is not from the soil or the bark where the liverwort was collected.

EXPERIMENTAL

^{1}H NMR: 400 or 600 MHz; ^{13}C NMR: 100 MHz (in $CDCl_3$ soln, TMS as int. standard). CC: silica gel 60 (70-230 mesh, Merck) and Sephadex LH-20 (Pharmacia). TLC: silica gel 60 F_{254} plates (Merck).

Symphyogyna brasiliensis (99.4 g) was collected in the area of IVIC, Caracas, Venezuela in April 1991. The voucher specimen is deposited in the herbarium of Tokushima Bunri University. After drying, the material was ground and extracted with Et_2O to afford a crude extract (650 mg). CC over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) afforded frs A, B and C. Fr. C was sepd by silica gel CC followed by prep. TLC to yield symphyogynolide (**1**) (14.6 mg).

Symphyogynolide (**1**). Oil; $[\alpha]_D$ $+38.3$ ($CHCl_3$; *c* 1.391); HRMS *m/z*: Found 334.2118; $C_{20}H_{30}O_4$ requires 334.2144; MS *m/z* (rel. int.): 334 $[M]^+$, 319, 279 (100), 263, 181, 147, 109, 55, 43; IR ν_{max} (FT) cm^{-1}: 1780, 1700, 1460, 1380, 1240; CD: $[\theta] = -1066.7$, $\Delta\varepsilon = -0.323$ (290 nm); ^{1}H and ^{13}C NMR: Table 1.

Unidentified *Frullania* species (No. 3122) (50 g) was collected in the area of IVIC, Caracas, Venezuela in April 1991. After drying, the material was ground and extracted with Et_2O to afford to crude extract (1.4 g). CC over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) followed by silica gel CC (hexane–EtOAc, gradient) afforded 6 frs. Fr. 2 was further sepd to give 3-methoxy-3′,4′-methylenedioxybibenzyl (**3**) [14, 15] (8.7 mg). From fr. 3, (+)-α-cyclocostunolide (**4**) [16] (2.4 mg) was isolated by HPLC. Fr. 4 was purified by recrystallization to afford methyl 3α-hydroxyolean-18-en-28-oate (**2**) (147.1 mg). Fr. 5 afforded rothin A acetate (**5**) [17] (6.9 mg) by silica gel CC and HPLC.

The other unidentified *Frullania* species (No. 3123) (55 g) was collected in the same area. After drying, the material was ground and extracted with Et_2O to afford a crude extract (1.56 g). CC over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) followed by silica gel CC (hexane–EtOAc, gradient) afforded 4 frs. From fr. 1, hinesene (**6**) [18] (18.1 mg) was isolated by prep. TLC. Fr. 2 was α-cyclocostunolide (**4**) (14.5 mg). HPLC separation of fr. 3 afforded rothin A acetate (**5**) (27.1 mg) and *ent*-cyclocolorenone (**7**) [16] (41.4 mg). Fr. 4 was further purified by silica gel CC and HPLC and gave a new farnesane type compound (**8**) (51.0 mg).

Methyl 3α-hydroxyolean-18-ene-28-oate (**2**). Mp 240–242° (hexane); $[\alpha]_D^{23}$ $+15.2$ ($CHCl_3$; *c* 1.7); HRMS (EI) *m/z*: Found 470.3747; $C_{31}H_{50}O_3$ requires 470.3760; EI-MS *m/z*: 470 $[M]^+$, 452, 437, 411, 393, 341, 273, 262, 249, 203, 189 (base), 175, 161, 135, 119, 109, 95, 81, 69, 55, 43; IR ν_{max} (FT) cm^{-1}: 3600, 1720, 1460, 1400, 1240; ^{1}H NMR (400 MHz); δ0.78 (3H, *s*), 0.83 (3H, *s*), 0.87 (3H, *s*), 0.93 (3H, *s*), 0.96 (3H, *s*), 0.97 (3H, *s*), 0.98 (3H, *s*), 3.40 (1H, *t*, $J = 2.8$, H-3), 3.68 (3H, *s*), and 5.11 (1H, *s*); ^{13}C NMR: Table 3.

Farnesane-type compound (**8**). HRMS: *m/z*: Found 234.1617; $C_{15}H_{22}O_2$ requires 234.1620; MS *m/z* 234 $[M]^+$, 219, 191, 166, 98 (100), 69, 41; IR ν_{max} (FT) cm^{-1}: 1780, 1750; ^{1}H NMR (400 MHz): δ5.86 (1H, *m*), 5.09 (2H, *m*), 4.74 (2H, *brs*), 1.69 (3H, *brs*), 1.62 (3H, *s*), 1.61 (3H, *s*); ^{13}C NMR (100 MHz): δ170.2, 137.6, 131.7, 124.0 (×2), 121.9, 115.6, 73.2, 39.6, 29.5, 28.7, 25.7 (×2), 17.7, 16.2.

Acknowledgements—We thank Dr T. Nakano, IVIC, Caracas, Venezuela, for his help in the collection of the liverworts, Dr R. Gradstein, University of Utrecht, The Netherlands, for identification of the liverworts, and Miss Y. Kan and Y. Okamoto for measurement of the 600 MHz NMR and mass spectra, respectively. This work is partly supported by a Grant-in-Aids for Cancer Research from the Ministry of Health and Welfare, Japan.

REFERENCES

1. Tori, M., Arbiyanti, H., Taira, Z. and Asakawa, Y. (1992) *Tetrahedron Letters* **33**, 4011.
2. Tori, M., Arbiyanti, H., Taira, Z. and Asakawa, Y. (1993) *Phytochemistry* **32**, 335.
3. Tori, M., Nakashima, K., Toyota, M. and Asakawa, Y. (1993) *Tetrahedron Letters* **34**, 3751.
4. Tori, M., Nakashima, K., Takeda, T. and Asakawa, Y. (1993) *Tetrahedron Letters* **34**, 3753.
5. Tori, M., Aoki, M. and Asakawa, Y. (1994) *Phytochemistry* **36**, 73.
6. Asakawa, Y. (1982) in *Progress in the Chemistry of Organic Natural Products* (Hertz, W., Grisebach, H. and Kirby, G. W., eds), Vol. 42, p. 1. Springer, Wien.
7. Asakawa, Y. (1990) in *Bryophytes: Their Chemistry and Chemical Taxonomy* (Zinsmeister, H. D. and Mues, R., eds), p. 369. Oxford University Press, Oxford.
8. Asakawa, Y. (1993) in *Bioactive Natural Products: Detection, Isolation and Structural Determination* (Colegate, S. M. and Molyneux, R. J., eds), p. 319. CRC Press, Florida.
9. Asakawa, Y., Matsuda, R., Takemoto, T., Hattori, S., Mizutani, M., Inoue, H., Suire, C. and Huneck, S. (1981) *J. Hattori Bot. Lab.* **50**, 107.
10. Asakawa, Y., Matsuda, R., Toyota, M., Suire, C., Takemoto, T., Inoue, H., Hattori, S. and Mizutani, M. (1981) *J. Hattori Bot. Lab.* **50**, 165.
11. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T. and Suire, C. (1979) *J. Hattori Bot. Lab.* **45**, 395.
12. Asakawa, Y., Toyota, M., Takeda, R., Suire, C. and Takemoto, T. (1981) *Phytochemistry* **20**, 725.
13. Wu, C.-L. (1992) *J. Chin. Chem. Soc.* **39**, 655.
14. Asakawa, Y., Takikawa, K. and Tori, M. (1987) *Phytochemistry* **26**, 1023.
15. Asakawa, Y., Matsuda, R. and Cheminat, A. (1987) *Phytochemistry* **26**, 1117.

16. Asakawa, Y., Matsuda, R., Toyota, M., Hattori, S. and Ourisson, G. (1981) *Phytochemistry* **20**, 2187.
17. Irvin, M. A. and Geissman, T. A. (1971) *Phytochemistry* **10**, 637.
18. Jakupovic, J., Grenz, M., Bohlmann, F., Wasshausen, D. C. and King, R. M. (1989) *Phytochemistry* **28**, 1937.
19. Takahashi, S. (1976) *Synthetic Commun.* **6**, 331.
20. Kobayashi, M. and Negishi, E. (1980) *J. Org. Chem.* **45**, 5223.
21. Corey, E. J. and Scmidt, G. (1980) *Tetrahedron Letters* **21**, 731.
22. Tius, M. A. and Takaki, K. S. (1982) *J. Org. Chem.* **47**, 3166.