*Phytochemistry*, Vol. 34, No. 3, pp. 751–753, 1993
Printed in Great Britain.

0031–9422/93 $6.00 + 0.00


# DITERPENOID CONSTITUENTS OF THE LIVERWORT *NARDIA SUBCLAVATA*

MASAO TOYOTA and YOSHINORI ASAKAWA

Institute of Pharmacognosy, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

(*Received* 4 *March* 1993)

**Key Word Index**—*Nardia subclavata*; Jungermanniaceae; Jungermanniales; Hepaticae; diterpene esters; bisditerpenoids; *ent*-16-kauren-14*R*-ol.

**Abstract**—The liverwort, *Nardia subclavata*, afforded two new diterpene esters, bis-[*ent*-16-kauren-14*R*-yl] malonate and a bisditerpene derived from the esterification of malonic acid by phytol and *ent*-16-kauren-14*R*-ol, in addition to perrottetin E, β-barbatene, (−)-kolavelool and *ent*-16-kauren-14*R*-yl hydrogen malonate. Their structures were established by chemical and spectral means. Perrottetin E and *ent*-16-kauren-14*R*-yl hydrogen malonate were the major constituents of this species.

## INTRODUCTION

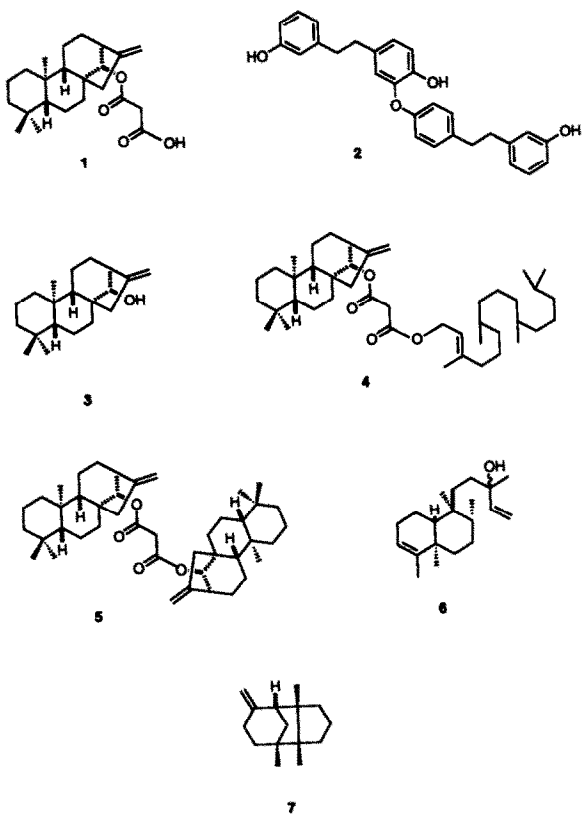
The epiphytic liverwort, *Nardia subclavata*, grows on moist soil or rock. The liverworts, including the Jungermanniales, are rich sources of terpenoids with a variety of carbon skeletons [1]. Previous phytochemical study of the genus *Nardia* S. Gray led to the isolation of *ent*-16-kauren-14*R*-yl hydrogen malonate from Scottish *N. scalaris* as its methyl ester [2] and of the *ent*-kaurene nardiin from Czechoslovakian *N. scalaris* [3]. Our previous work resulted in the identification of 11-hydroxykauran-15-one, together with limonene and myrcene from *N. compressa* [4], and α- and β-barbatene, β-bisabolene and cuparene from *N. scalaris* [5] by GC-mass spectral analysis.

Classification of liverworts belonging to the Jungermanniaceae, a family rich in diterpenes [1], is very difficult and study of their chemical constituents is necessary. For this reason, we have been investigating the chemosystematics of the liverworts [1, 4, 5]. Here we report on the isolation and structure elucidation of two new diterpene esters from Japanese *N. subclavata*, a species which has not yet been investigated phytochemically.

## RESULTS AND DISCUSSION

The ether extract of *N. subclavata* was chromatographed on silica gel and Sephadex LH-20 to give *ent*-16-kauren-14*R*-yl hydrogen malonate (**1**) and perrottetin E (**2**) [6] as major constituents, together with β-barbatene (**7**) [7] and a mixture of diterpenoids. Further purification of the mixture by MPLC yielded two new diterpenic esters **4** and **5**, together with *ent*-16-kauren-14*R*-ol (**3**) and (−)-kolavelool (**6**) [8].

The spectral data of compound **2** and its methyl ether derivative were in agreement with those of previous reported values [6]. Compound **3** and its pyridinium chlorochromate oxidation product were characterized by comparing spectral data and physical constants with literature values [2]. Hydrolysis of **1** with 5% methanolic potassium hydroxide gave an alcohol and malonic acid. The spectral data and physical constants of the alcohol were identical to those of *ent*-16-kauren-14*R*-ol (**3**). This is the first report of the isolation of *ent*-16-kauren-14*R*-yl



hydrogen malonate (1) in nature, although it has been isolated from *N. scalaris* as its methyl ester [2]. The spectral data are described in the Experimental, as there is no report of these data in the literature.

The structures of **4** and **5** were deduced by comparing their spectral data with those of **1**. Compound **4**, an oil, produced the same bright pink colouration as compound **1** with Godin reagent on TLC plates. The EI mass spectrum of **4** gave a molecular ion peak at *m/z* 652 and the same base peak at *m/z* 270 as that of **1**. Whereas the carboxylic acid absorption was missing in the IR spectrum of **4**, the presence of two ester carbonyl (1750 and 1734 $cm^{-1}$) groups was confirmed. Compound **4** gave signals in its $^1H$ and $^{13}C$ NMR spectra for two ester carbonyl groups ($\delta_C$ 166.7 and 166.3), an exomethylene group [$\delta_H$ 4.85 (2H), $\delta_C$ 105.3 and 152.3] and a trisubstituted double bond ($\delta_H$ 5.33, $\delta_C$ 117.5 and 143.2). Hydrolysis of **4** with 5% methanolic potassium hydroxide gave two alcohols and malonic acid. The spectral data and physical constants of the alcohols were identical to those of **3** and phytol, respectively. Accordingly, the structure of the bisditerpenoid was formulated as **4**.

The $^1H$ and $^{13}C$ NMR spectra of **5**, $M_r$ 644, were almost identical to those of **1**, except for a decrease in the intensity of the signals for an isolated methylene group in the malonic acid moiety. Hydrolysis of **5** with 5% methanolic potassium hydroxide gave an alcohol whose spectral data were in agreement with those of **3**. While the hydrolysis of **4** gave **3** and phytol, hydrolysis of **5** only gave **3**, although both reactions gave malonic acid. Consideration of these chemical and spectral data led to the conclusion that the structure of **5** was a bisditerpenoid, derived from the esterification of malonic acid and two units of *ent*-16-kauren-14*R*-ol.

Malonates are rare in general. A few examples are known, such as the *Calceolaria densifolia* (Scrophulariaceae) bisditerpene derived from the esterification of malonic acid by two units of *ent*-9-epi-labda-8(17),(12*Z*), 14-trien-19-ol [9], and the malonate of polyporenic acid A from a fungal source [10].

During this investigation, different species of *Nardia* S. Gray were analysed by TLC. The constituents of *N. subclavata* and *N. scalaris* were very similar, except for the presence of larger proportions of perrottetin E (**2**) in the former species. However, *N. assamica* was completely different from the other species in its constituents.

## EXPERIMENTAL

*General.* Mps: uncorr; TLC: silica gel precoated glass plates with *n*-hexane–EtOAc (1:1 and 4:1). Detection was with Godin reagent [11]. CC: silica gel 60 (40–63 $\mu$m). The solvent $CH_2Cl_2$–MeOH (1:1) was used for CC on Sephadex LH-20.

*Spectral data.* NMR: 100 MHz for $^{13}C$ and 400 MHz for $^1H$; EIMS: 70 eV.

*Plant material. Nardia subclavata* (34 g) was collected in October 1992 at Kisawason, Tokushima. *Nardia scalaris* subsp. *harae* (79 g) and *N. assamica* (44 g) were collected in November 1992 at Kamiyama, Tokushima. The voucher specimens (# 92048, 92075 and 92062) are deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

*Extraction and isolation.* The liverworts were dried for 1 week, after removal of impurities such as other species under a stereo microscope with tweezers, ground mechanically and then extracted with $Et_2O$ for 1 month. The $Et_2O$ extract (715.5 mg) of *N. subclavata* was chromatographed on silica gel using a *n*-hexane–EtOAc gradient, giving 10 frs (I–X). The $^1H$ NMR and GC-MS of fr. II (23.8 mg; 3.3% of the total extract) were identical to those of $\beta$-barbatene (**7**) [7]. Fr. IX (68.6 mg) was rechromatographed on Sephadex LH-20 to give perrottetin E (**2**) (57.2 mg; 8.0%) [6]. Further purification of fr. X (72 mg) by CC on Sephadex LH-20 afforded *ent*-16-kauren-14*R*-yl hydrogen malonate (**1**) (55.6 mg; 7.8%). Fr. V (65.4 mg) was rechromatographed on Sephadex LH-20 to give *ent*-16-kauren-14*R*-ol (**3**) (11.4 mg; 1.6%) and a mixt. containing **6**. The mixt. was repurified by MPLC on silica gel using *n*-hexane–EtOAc (9:1), affording (−)-kolavelool (**6**) (22.3 mg; 3.1%). Fr. IV (111.9 mg) was further chromatographed on Sephadex LH-20 to give a mixt. of **4** and **5**. The mixt. was further purified by MPLC on silica gel using *n*-hexane–EtOAc (19:1) to give the new diterpenic esters **4** (14.8 mg; 2.1%) and **5** (8.2 mg; 1.2%), respectively. Compound **1**: $[\alpha]_D^{21}$ −37.8° ($CHCl_3$; *c* 2.78), IR $\nu_{max}$ (neat) $cm^{-1}$: 3400–3000, 1740, 1717, 1451, 1318, 1148, 1001, 878, 760; $^1H$ NMR ($CDCl_3$): $\delta$ 0.81, 0.85 and 1.07 (each 3H, *s*), 2.10 (1H, *br d*, *J* = 17 Hz), 2.31 (1H, *ddd*, *J* = 3, 3, 17 Hz), 2.70 (1H, *br s*), 3.44, (2H, *s*), 4.87 (2H, *br s*), 5.46(1H, *br s*); $^{13}C$ NMR ($CDCl_3$): $\delta$ 17.9, 21.6 and 33.6 (Me), 17.2, 18.7, 19.7, 32.7, 33.1, 40.2, 40.9, 41.9 and 45.6 ($CH_2$), 49.0, 56.2 and 59.1 (CH), 33.2, 39.3 and 48.3 (C), 81.7 (O−CH), 105.6 (C=$\underline{C}H_2$), 151.9 ($\underline{C}$=$CH_2$), 166.7 and 171.3 (C=O); EIMS *m/z* (rel. int.): 374 $[M]^+$ (0.6), 359 $[M-Me]^+$ (1), 356 $[M-18]^+$ (1), 315 $[M-CH_2COOH]^+$ (3), 270 (100), 255 (30), 131 (32), 123 (38), 119 (32), 109 (32), 105 (40), 95 (43), 93 (36), 91 (60), 87 (50), 81 (56), 79 (46), 69 (73), 67 (48), 55 (66). Compound **4**: $[\alpha]_D^{21}$ −31.1° ($CHCl_3$; *c* 0.74), IR $\nu_{max}^{neat}$ $cm^{-1}$: 1750, 1734, 1661, 1460, 1368, 1267, 1146, 1005, 876; $^1H$ NMR ($CDCl_3$): $\delta$ 0.81, 0.84 and 1.07 (each 3H, *s*), 1.69 (3H, *br s*), 0.84, 0.85 (× 2) and 0.87 (each 3H, *d*, *J* = 6.4 Hz), 2.07 (1H, *br d*, *J* = 17 Hz), 2.31 (1H, *ddd*, *J* = 3, 3, 17 Hz), 2.68 (1H, *br s*), 3.36 (2H, *s*), 4.64 (2H, *d*, *J* = 7 Hz), 4.85 (2H, *br s*), 5.33 (1H, *br t*, *J* = 7 Hz), 5.40 (1H, *br s*); $^{13}C$ NMR ($CDCl_3$): $\delta$ 16.4, 18.0, 19.8 (× 2), 21.6, 22.6, 22.7 and 33.6 (Me), 17.3, 18.7, 19.7, 24.5, 24.8, 25.1, 32.7, 33.2, 36.7, 37.3, 37.4, 37.5, 39.4, 39.9, 40.3, 41.9, 42.0 and 45.7 ($CH_2$), 28.0, 32.7, 32.8, 49.0, 56.3 and 59.1 (CH), 33.2, 39.3 and 48.4 (C), 62.4 (O-$CH_2$), 81.0 (O-CH), 105.3 (C=$\underline{C}H_2$), 117.5 (C=$\underline{C}$H), 143.2 ($\underline{C}$=CH), 152.3 ($\underline{C}$=$CH_2$), 166.7 and 166.3 (C=O); EIMS *m/z* (rel. int.): 652 $[M]^+$ (0.4), 548 (3), 270 (100), 252 (39), 123 (66), 119 (35), 109 (37), 97 (37), 96 (34), 95 (89), 83 (52), 82 (93), 81 (89), 79 (31), 71 (43), 69 (81), 68 (88), 67 (44), 57 (82), 55 (77). Compound **5**: mp 197–198°, $[\alpha]_D^{21}$ −53.5° ($CHCl_3$; *c* 0.41); IR $\nu_{max}^{neat}$ $cm^{-1}$: 1746, 1730, 1661, 1451, 1316, 1267, 1186, 1146, 1005, 876, 758; $^1H$ NMR ($CDCl_3$): $\delta$ 0.81, 0.84 and 1.07 (each 6H, *s*), 2.06 and 2.32 (each 2H, *br d*, *J* = 17 Hz), 2.69 (2H, *br s*), 3.36 (2H, *s*), 4.85 (4H, *br s*), 5.40 (2H, *br s*); $^{13}C$ NMR

($CDCl_3$): $\delta$ 17.9, 21.6 and 33.6 (Me), 17.2, 18.7, 19.8, 32.7, 33.2, 40.3 (x 2), 41.9 and 45.7 ($CH_2$), 48.9, 56.2 and 59.1 (CH), 33.2, 39.3 and 48.3 (C), 81.0 (O–CH), 105.3 (C $=\underline{C}H_2$), 152.3 ($\underline{C}=CH_2$), 166.4 (C=O); EIMS *m/z* (rel. int.): 644 $[M]^+$ (3), 540 (3), 271 (90), 270 (100), 146 (15), 145 (16), 133 (17), 119 (26), 106 (15), 95 (17), 91 (18), 81 (20), 69 (26), 55 (20).

*Methylation of* **2** *with* MeI. Compound **2** (57 mg) in $Me_2CO$ (3 ml) was methylated with MeI (4 ml) in the presence of dry $K_2CO_3$ at reflux temp. for 10 hr. The reaction mixt. was filtered and the filtrate evapd. The resulting product was purified through a small column packed with silica gel using $Et_2O$ as solvent to give a trimethyl ether (43.6 mg). The $^1H$ NMR and EIMS data were identical to those of perrottetin E methyl ether [6].

*Oxidation of* **3** *with pyridinium chlorochromate ( PCC).* To PCC (17 mg) in $CH_2Cl_2$ (3 ml) was added **3** (3.3 mg) in $CH_2Cl_2$ (0.5 ml) and the mixt. was stirred for 2 hr at room temp. The resulting mixt. was filtered and the solvent removed to yield a ketone (1.7 mg). Its $^1H$ NMR and IR data were identical to those of literature values [2].

*Basic hydrolysis of* **1**, **4** *and* **5**. A soln of **1** (55 mg), **4** (14 mg) and **5** (8 mg) in 5% methanolic KOH (1 ml) was refluxed for 30 min. After evapn, $H_2O$ (3 ml) was added and the soln extracted with $Et_2O$. The $Et_2O$ layer was washed with $H_2O$, dried over $Na_2SO_4$ and evapd to give alcohol (28.7, 3 and 3 mg) and phytol (3 mg) from **4**. The $^1H$ NMR data were identical to those of authentic samples. The aq. layer of the reaction mixt. was acidified with aq. 5% HCl, lyophilized, then extracted with MeOH and evapd to yield an acid (5, 1 and 1 mg, respectively) whose $^1H$ and $^{13}C$ NMR spectra and GC-MS of its TMS derivative were identical to those of malonic acid.

*Acknowledgment*—We thank Dr M. Mizutani (Hattori Botanical Laboratory, Nichinan, Miyazaki, Japan) for the identification of the liverworts. This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.

## REFERENCES

1. Asakawa, Y. (1982) in *Prog. Chem. Org. Nat. Prod.* **42**, 1.
2. Connolly, J. D., Harrison, L. J., Phillips, W. R. and Rycroft, D. S. (1984) *Chem. Res. (S)* 94.
3. Benes, I., Vanek, T. and Budesinsky, M. (1982) *Collect. Czech. Chem. Commun.* **47**, 1873.
4. Asakawa, Y., Suire, C., Toyota, M., Tokunaga, N., Takemoto, T., Hattori, S. and Mizutani, M. (1980) *J. Hattori Bot. Lab.* **48**, 285.
5. Asakawa, Y., Matsuda, R., Toyota, M., Suire, C., Takemoto, T., Inoue, H., Hattori, S. and Mizutani, M. (1981) *J. Hattori Bot. Lab.* **50**, 165.
6. Toyota, M., Tori, M., Takikawa, K., Shiobara, Y., Kodama, M. and Asakawa, Y. (1985) *Tetrahedron Letters* **26**, 6097.
7. Connolly, J. D., Harding, A. E. and Thornton, I. M. S. (1972) *J. Chem. Soc. Chem. Commun.* 1320.
8. Misra, R., Pandey, R. C. and Dev, S. (1979) *Tetrahedron* **35**, 985.
9. Garbarino, J. A. and Molinari, A. (1992) *J. Nat. Prod.* **55**, 744.
10. Bryce, T. A., Campbell, I. M. and McCorkindale, N. J. (1976) *Tetrahedron* **23**, 3427.
11. Godin, P. (1954) *Nature* **174**, 134.