Phytochemistry, Vol. 28, No. 9, pp. 2507–2509, 1989.
Printed in Great Britain.

0031–9422/89 $3.00+0.00
Maxwell Pergamon Macmillan plc.

A CLERODANE-TYPE DITERPENIC ACID FROM THE LIVERWORT *JUNGERMANNIA INFUSCA*

MASAO TOYOTA, FUMIHIRO NAGASHIMA and YOSHINORI ASAKAWA

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

(*Received* 3 *January* 1989)

Key Word Index—*Jungermannia infusca*; Jungermanniales; Hepaticae; clerod-3,13(16),14-trien-17-oic acid; (+)-isoabienol; gomeraldehyde; 13-epigomeraldehyde; clerodane and labdane-type diterpenoids; superoxide release inhibitory activity.

Abstract—A new clerodane-type diterpenoid, possessing superoxide release inhibitory activity has been isolated from the liverwort *Jungermannia infusca*, together with three known diterpenoids and its structure characterized as clerod-3,13(16)-14-trien-17-oic acid by 2D NMR spectroscopy.

INTRODUCTION

The liverworts belonging to the Jungermanniaceae are rich sources of sesqui- and diterpenoids [1]. As part of a chemosystematic study [2] and search for biologically active substances [3–11], we have investigated *Jungermannia infusca* and isolated a new clerodane-type diterpenic acid (**1**) possessing potent superoxide release inhibitory activity, along with the previously known diterpenoids (**5, 6**) [12] and **7** [13].

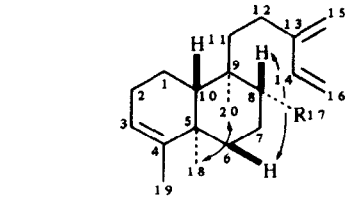
RESULTS AND DISCUSSION

Silica gel and Sephadex LH-20 column chromatography of the ether extract of *J. infusca* resulted in the isolation of compound **1** and the previously known diterpenoids (**5, 6**) [12] and (**7**) [13]. Compound **1**, mp 138–140°, had the molecular formula $C_{20}H_{30}O_2$ (*m/z* 302.2227). The acidic nature of **1** was deduced from the long tailing spot on TLC and the broad absorption band between 3 000 and 3 500 cm^{-1} in the IR spectrum and the formation of a mono methyl ester (**2**) (δ_H 3.65, *s*, 3H). The UV spectrum suggested the presence of a conjugated double bond system (λ_{max} 226 nm). The ^{13}C NMR spectrum of **1** (Table 1) exhibited the signals due to six sp^2 carbons and a carboxylic carbon, having six degree of unsaturation. These spectral data disclosed that **1** might be a bicyclic skeleton. The ^1H–^1H COSY spectrum of **1** showed the protons (H-7α and 7β) at δ 1.97 and 1.72 were correlated with the proton (H-8) at δ 2.56 and the protons (H-6α and 6β) at δ 1.82 and 1.20. Furthermore, the protons (H-12) at δ 2.09 and 2.33 were correlated with the protons (H-11) at δ 1.65 and 1.53. The long range ^1H–^{13}C COSY spectrum (Table 2) of **1** indicated that the carboxyl carbon was correlated with the quaternary carbon (C-9) and the tertiary methyl carbon (C-20). Moreover, one of the tertiary methyl groups (H-20; δ0.95) was correlated with two methine carbons (C-8 and C-10) and also with the methylene carbon (C-11). Another tertiary methyl group (H-18; δ 1.07) was correlated with the quaternary sp^2 carbon (C-4), the methine carbon (C-10) and with the methylene carbon (C-6). Furthermore, the protons (H-3; δ 5.22) on the trisubstituted double bond were correlated with the quaternary carbon (C-5) which was correlated with the vinyl methyl groups (H-19; δ 1.61). On the basis of the above spectral evidence, the structure of **1** was assumed to be a clerodane-type diterpenoid. As the diterpene with the clerodane-skeleton, kolavenic acid (**4**) [14, 15] which was isolated from the root of *Solidago* species (collected in Tokushima, 1986) was completely assigned for the signals of the protons and carbons by the extensive 2D COSY NMR spectral techniques. Com-

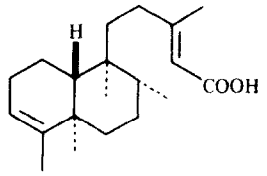
Table 1. ^{13}C NMR spectral data of **1** and **4** (100 Mz, $CDCl_3$)*

C	1	4
1	18.0	18.3
2	26.6	26.9
3	120.7	120.4
4	143.8	144.4
5	37.9	38.2
6	35.6	36.8
7	21.7	27.5
8	49.1	36.3
9	38.9	38.3
10	46.4	46.5
11	39.5	36.3
12	24.8	35.0
13	147.1	164.6
14	139.0	114.8
15	113.2	172.1
16	115.9	19.5
17	181.0	16.0
18	20.1	20.0
19	18.0	18.0
20	20.1	18.3

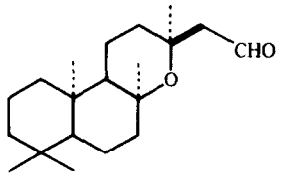
*All assignments were confirmed by INEPT, ^1H–^{13}C COSY and ^1H–^{13}C long range COSY spectra.



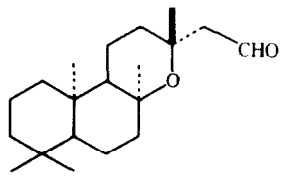
1 R = COOH NOE (↷)
2 R = COOMe
3 R = CH_2OH



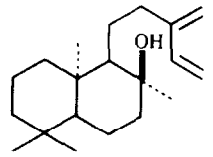
4



5



6



7

Table 2. ^1H–^{13}C correlation of **1**

^1H	^{13}C	^1H	^{13}C
H-3	C-5	H-18	C-4
H-6	C-10		C-5
H-8	C-9		C-6
	C-17		C-10
	C-20	H-19	C-3
H-10	C-1		C-4
	C-18		C-5
	C-20	H-20	C-8
H-11	C-8		C-9
H-12	C-13		C-10
	C-16		C-11
H-15	C-13		

parison of ^1H and ^{13}C NMR spectra of **1** with those of **4** showed only minor difference for skeletal proton and carbon signals and differed only in the signals due to the side chain. On the whole, the structure of **1** was established to be clerod-3,13(16)-14-trien-17-oic acid. The relative stereochemistry of **1** was established by the difference NOE experiment. Compound **1** showed the NOEs between (i) H-18 and H-19 and (ii) H-6 and H-8.

This is the second record of co-occurrence of the clerodane- and labdane-type diterpenoids in the liverworts [16]. Compound **1** inhibited the release of superoxide from rabbit PMN at IC_{50} 15 μg/ml and from guinea pig macrophage at IC_{50} 2 μg/ml.

EXPERIMENTAL

Mps: uncorr. The solvents used for spectral determinations were TMS-$CDCl_3$ [^1H NMR (400 MHz) and ^{13}C NMR (100 MHz)]; $CHCl_3$ (IR); EtOH (UV); $CHCl_3$ ($[\alpha]_D$). A mixed solvent of MeOH and $CHCl_3$ (1:1) was used for Sephadex LH-20 column chromatography. TLC was carried out as previously reported [17].

Plant material. Jungermannia infusca was collected in Azumadani, Tokushima, in May 1986 and identified by Dr M. Mizutani. The voucher specimen was deposited in the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. The fresh *J. infusca* (354 g) was extracted with Et_2O for 2 months. The crude extract (6.50 g), after removal of the solvent, was chromatographed on silica gel using *n*-hexane and EtOAc gradient to divide into seven fractions. Fr. 5 (665.8 mg) was further chromatographed on Sephadex LH-20 to give gomeraldehyde (**5**) (57.4 mg), 13-epigomeraldehdye (**6**) (32.1 mg) [12] and (+)-isoabienol (**7**) [13] whose spectral data were identical to those reported in the references. Fr. 6 (311.9 mg) was rechromatographed on Sephadex LH-20 to afford **1** (68.6 mg); mp 138–140°; $[\alpha]_D$ −43.6° (*c* 1.26); UV λ_{max} nm (log ε): 226 (3.17); IR ν_{max} cm^{-1}: 3500–3000, 1700; ^1H NMR δ: 0.95 (3H, *s*, H-20), 1.07 (3H, *s*, H-18), 1.20 (1H, *m*, H-6), 1.53 (1H, *m*, H-11), 1.61 (3H, *s*, H-19), 1.65 (1H, *m* H-11), 1.72 (1H, *m*, H-7), 1.82 (1H, *m*, H-6), 1.97 (1H, *m*, H-7), 2.09 (3H, *m*, H-2 and H-12), 2.33 (1H, *ddd*, *J* = 13.7, 13.7, 4.4, H-12), 2.55 (1H, *dd*, *J* = 13.2, 4.1, H-8), 5.00, 5.01 (each 1H, *s*, H-16), 5.05 (1H, *d*, *J* = 10.7, H-15), 5.22 (1H, *br s*, H-3), 5.30 (1H, *d*, *J* = 17.6, H-15), 6.36 (1H, *dd*, *J* = 17.6, 10.7, H-14); ^{13}C NMR (see Table 1); HRMS: found 302.2227 $C_{20}H_{30}O_2$, require 302.2246; EIMS *m/z* (rel. int.): 302 $[M]^+$ (17), 286 (22), 273 (20), 259 (7), 241 (26), 219 (43), 203 (31), 189 (20), 173 (87), 159 (34), 145 (30), 133 (62), 119 (75), 107 (94), 95 (100), 81 (78), 69 (61).

Methylation of **1**. Compound **1** (35 mg) was methylated with CH_2N_2 at room temp. to give a mono methyl ester (**2**) (32 mg). ^1H NMR δ: 0.90, 1.07, 1.60 (each 3H, *s*), 2.32 (1H, *ddd*, *J* = 13.4, 13.4, 4.2), 2.55 (1H, *ddd*, *J* = 12.7, 12.7, 3.4), 3.65 (3H, *s*, COOMe), 4.99, 5.00 (each 1H, *s*), 5.07 (1H, *d*, *J* = 10.7), 5.21 (1H, *br s*), 5.29 (1H, *d*, *J* = 17.6), 6.37 (1H, *dd*, *J* = 17.6, 10.7), which was reduced by $LiAlH_4$ (10 mg) in Et_2O (3 ml). Work-up as usual gave a primary alcohol (**3**) (11 mg). IR ν_{max} cm^{-1}: 3600, 2925, 1375; ^1H NMR δ: 0.75, 1.03, 1.61 (each 3H, *s*), 2.18 (1H, *ddd*, *J* = 13.2, 13.2, 4.4), 3.36 (1H, *dd*, *J* = 10.7, 3.4), 3.84 (1H, *dd*, *J* = 10.7, 3.9), 4.99 (2H, *s*), 5.06 (1H, *d*, *J* = 11.7), 5.21 (1H, *br s*), 5.23 (1H, *d*, *J* = 17.6), 6.37 (1H, *dd*, *J* = 17.6, 11.7); ^{13}C NMR δ: 17.9, 18.0, 19.9 (*q*), 17.9, 22.0, 24.8, 26.8, 36.2, 37.4, 64.3, 113.1, 115.7 (*t*), 44.3, 46.5, 120.6, 139.0 (*d*), 38.0, 38.3, 144.3, 147.3 (*s*); EIMS *m/z* (rel. int.): 288 $[M]^+$ (3), 273 (3), 257 (4), 205 (10), 187 (9), 175 (6), 159 (11), 145 (15), 133 (12), 119

(25), 107 (25), 95 (37), 81 (27), 67 (16), 55 (24), 41 (27), 28 (16), 18 (100).

Acknowledgements—We thank Dr Y. Fukuyama (Tokushima Research Institute, Otsuka Pharmaceutical Co. Ltd) for carrying out biological screening and Dr M. Mizutani (The Hattori Botanical Laboratory, Nichinan, Japan) for his identification of the liverwort.

REFERENCES

1. Asakawa, Y. (1982) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Griesebach, H. and Kirby, G. W., eds), Vol. 42, p. 1. Springer, Wien.
2. Toyota, M., Nagashima, F. and Asakawa, Y. (1988) *Phytochemistry* **27**, 2603.
3. Asakawa, Y. (1981) *J. Hattori Bot. Lab.* **50**, 123.
4. Asakawa, Y. (1984) *J. Hattori Bot. Lab.* **56**, 215.
5. Asakawa, Y. (1984) *Rev. Latinoamer. Quim.* **14**, 105.
6. Asakawa, Y. (1984) *Kagaku to Seibutsu* **22**, 495.
7. Asakawa, Y. (1985) *Seiyakukojo* **5**, 284.
8. Asakawa, Y. (1987) *Farumashia* **23**, 455.
9. Asakawa, Y. (1988) in *Studies in Natural Products Chemistry* (Rahman, A., ed.), Vol. 2, p. 277. Elsevier, Amsterdam.
10. Asakawa, Y. (1988) in *Physiology and Biochemistry of Development of Bryophytes* (Chopra, R. N. and Bhatla, S. C., eds). CRC Press, Florida (in press).
11. Asakawa, Y. (1988) in *Annual Proceedings of the Phytochemical Society of Europe* (Zinsmeister, H. D. and Mues, R., eds), Oxford University Press, Oxford (in press).
12. Gonzalez, A. G., Fraga, B. M., Hernandez, M. G., Larruga, F. and Luis, J. G. (1975) *Phytochemistry* **14**, 2655.
13. Chirkova, M. A., Gorbunova, A. E., Lisina, A. I. and Pentegova, V. A. (1966) *Khim. Prirodn. Soedin.* 99.
14. Misra, R., Pandey, R. C. and Dev, S. (1964) *Tetrahedron Letters* 3751.
15. Anthonsen, T. and McCrindle, R. (1969) *Acta Chem. Scand.* **23**, 1068.
16. Wu, C.-L. and Asakawa, Y. (1987) *Phytochemistry* **26**, 940.
17. Asakawa, Y., Tori, M., Takikawa, K., Krishnamurty, H. and Kar, S. K. (1987) *Phytochemistry* **26**, 1811.

Phytochemistry, Vol. 28, No. 9, pp. 2509–2511, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00
Maxwell Pergamon Macmillan plc.

SAPOGENINS FROM *SOLANUM MERIDENSE*

BRIGITA DE VALERI and ALFREDO USUBILLAGA*

Chemistry Department, Faculty of Sciences, University of Los Andes, Mérida, Venezuela; *Instituto de Investigacion, Faculty of Pharmacy, University of Los Andes, Mérida, Venezuela

(*Received in revised form* 7 *February* 1989)

Key Word Index—*Solanum meridense*; Solanaceae; steroidal sapogenins; (25*R*)-3β-acetoxy-5α-spirostan-6-one; chlorogenone; chlorogenin; diosgenin; sitosterol.

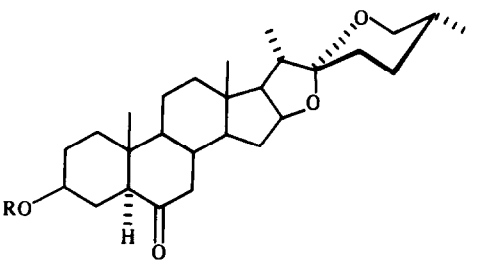
Abstract—In addition to chlorogenin, chlorogenone, diosgenin, and sitosterol a new sapogenin has been isolated from the green berries of *Solanum meridense*. Its structure was established by spectral data and chemical degradation as (25*R*)-3β-acetoxy-5α-spirostan-6-one.

INTRODUCTION

There are no previous reports on the chemical constituents of *Solanum meridense* Bitter et Pittier. This small tree is widely distributed around Mérida and its fruits are known for their foam forming properties, indicating the presence of sapogenins.

RESULTS AND DISCUSSION

Dried and powdered berries were treated as described in the Experimental to yield a chloroform extract. Repeated preparative TLC over silica gel plates yielded



1a R = Ac
1b R = H