Pergamon

Phytochemistry, Vol. 48, No. 7, pp. 1173–1177, 1998

Printed in Great Britain
0031–9422/98 $19.00+0.00

PII: S0031-9422(97)00786-3

DITERPENES AND STEROLS FROM *NEOBOUTONIA MELLERI*

WEIMIN ZHAO, JEAN-LUC WOLFENDER, S. MAVI† and KURT HOSTETTMANN*

Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015 Lausanne, Switzerland; † National Herbarium and National Botanic Garden, Causeway, Harare, Zimbabwe

(*Received* 12 *August* 1997)

Key Word Index—*Neoboutonia melleri*; Euphorbiaceae; diterpenes; sterols; mellerin A and B.

Abstract—Two new diterpenes (**1** and **2**) named mellerin A and B along with three known sterols (**3**–**5**) were isolated from the leaves of *Neoboutonia melleri* (Euphorbiaceae). Their structures were identified on the basis of chemical and spectroscopic methods. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Species of the family Euphorbiaceae have been the source of many biologically active compounds. A considerable attention has been given to their skin-irritant diterpene esters [1, 2]. As part of our phytochemical investigations of African plants, we have studied the lipophilic extract of the leaves of *Neoboutonia melleri* (Euphorbiaceae) collected in Zimbabwe. As a result, two new diterpenes (**1** and **2**) named mellerin A and B along with (24*R*)-24-ethylcholesta-3β,5α,6β-triol (**3**), 7β-hydroxysitosterol (**4**) and β-sitosterol (**5**) were isolated from the dichloromethane extract of the plant. Their structures were established with the help of chemical and spectroscopic methods. To our best knowledge, this is the first report on the chemical components of plants from the genus *Neoboutonia*.

RESULTS AND DISCUSSION

The leaves of *Neoboutonia melleri* (1.6 kg) were ground under liquid nitrogen and then extracted with dichloromethane and methanol, successively. The dichloromethane extract (60 g) was chromatographed on a silica gel column with a petroleum ether–acetone (4:1 → 0:1) gradient and then methanol to give seven fractions. The fractions were further filtered through Sephadex LH-20 columns with chloroform–methanol (1:1), and subjected to chromatographies on RP-18 Lobar columns with methanol–water (9:1) and on silica gel columns with a chloroform–acetone gradient (5:1 → 1:1) to give compounds **1** (150 mg), **2** (200 mg), **3** (8 mg), **4** (10 mg) and **5** (500 mg).

Compound **1** was obtained as a colourless gum. Its positive ion mode D/CI mass spectrum gave quasimolecular ion adducts at *m/z* 492 $[M+NH_4]^+$ and 475 $[M+H]^+$, and a quasimolecular ion peak $[M+H]^-$ was observed at *m/z* 473 in the negative ion mode D/CI mass spectrum, which indicated its molecular weight to be 474. The ^{13}C NMR and DEPT spectra of **1** exhibited 28 carbon signals as five methyls, nine methylenes, six methines and eight quaternary carbons. Among them were two carbonyl carbon signals at δ 209.5 and 175.9 ppm and four olefinic carbon signals at δ 161.2 (*d*), 140.1 (*s*), 132.7 (*s*) and 130.1 (*d*) ppm. The UV spectrum of **1** exhibited a maximum absorption peak at λ 235 nm, which suggested the existence of conjugated double bonds. According to the ^{13}C NMR, DEPT and mass spectra, the molecular formula of **1** was deduced to be $C_{28}H_{42}O_6$.

In the ^1H NMR spectrum of **1**, five methyls at δ 1.70, 1.18, 1.07, 0.86 and 0.85 ppm along with two olefinic proton signals at δ 7.56 and 5.65 ppm were identified. Besides, eight proton signals overlapping between δ 1.25 and 1.30 ppm were found. Analysis of DQF-COSY, HMQC and HMBC spectra of **1** indicated the existence of an octanyl ester group in the structure of **1**. This was also confirmed by the mass fragments at *m/z* 330 $[M-CH_3(CH_2)_6COOH-H_2O+NH_4]^+$ and 348 $[M-CH_3(CH_2)_6COOH+NH_4]^+$ in the positive ion mode D/CI mass spectrum. The other part of the molecule contained twenty carbons and was suggested to be a diterpene. Compound **1** was therefore supposed to be a diterpenic ester of an octanoic acid.

Further analysis of the DQF-COSY and TOCSY spectra of **1** revealed the existence of the following fragments: CH_3—C=CH—CH—, CH_3—CH—CH_2— and —CH—CH—CH=C(—CH_2—)—CH_2O—. The connectivities of the above fragments and other quaternary carbons was determined on the basis of ^1H-^{13}C long range correlation signals in the HMBC spectrum of **1** as shown in Fig. 1. These data

* Author to whom correspondence should be addressed.

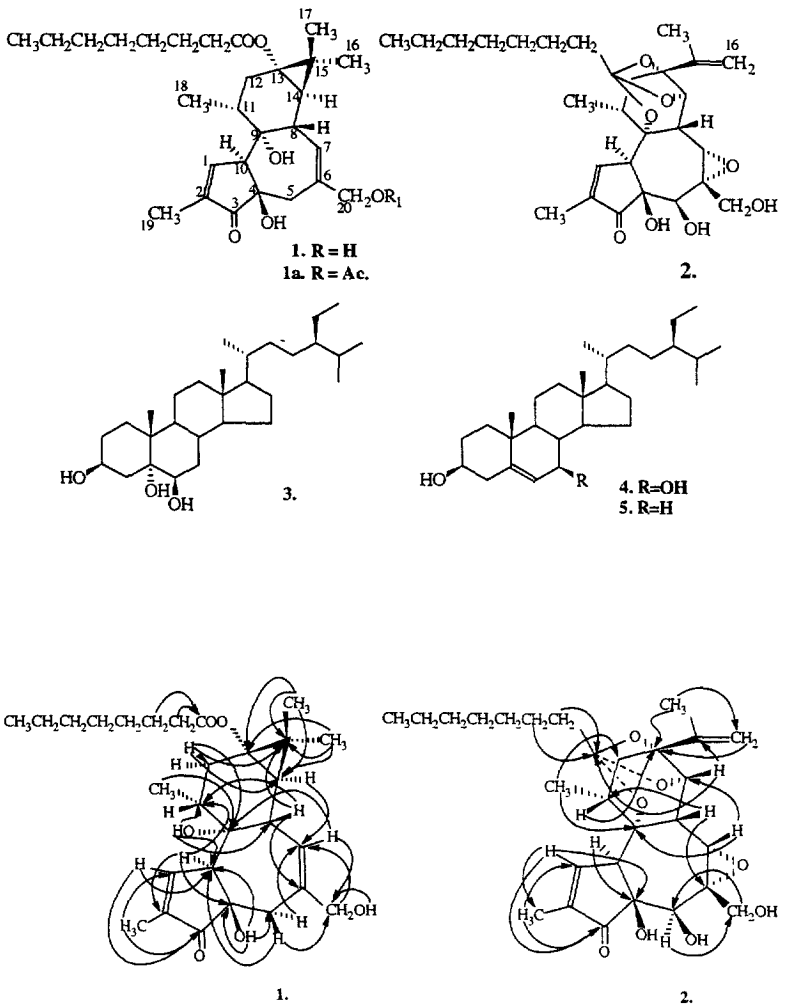

Fig. 1. Main ^{1}H-^{13}C long range correlation signals observed in HMBC spectra of **1** and **2**.

suggested that compound **1** possessed a tigliane-type skeleton. This type of diterpenes were found before in several Euphorbiaceae species [3, 4]. In order to determine the position of the octanyl group using NMR methods, the chemical shifts of the hydroxy proton signals have to be characterized. Therefore, NMR spectra of **1** were measured again in d_6-DMSO. When the ^{1}H NMR spectrum was measured at 26°C, one hydroxy proton was found at δ 5.61 along with the other two hydroxyl proton signals overlapping at δ 4.70 ppm. This render their position assignments difficult in the HMBC spectrum. When the temperature raised to 35°C, the two overlapped hydroxyl proton signals could be differentiated at δ 4.67 (*s*) and 4.66 (*t*, 5.5), respectively. Therefore, all the following spectra were measured at 35°C. The ^{1}H and ^{13}C NMR spectra of **1** in d_6-DMSO were still similar to those measured in $CDCl_3$, both proton and carbon signals could thus be easily assigned using DQF-COSY and HMQC spectra. In the HMBC spectrum of **1**, ^{1}H-^{13}C long range correlation signals were found among the three hydroxy proton signals and their neighbouring carbon signals as shown in Fig. 1. Therefore, the hydroxy group at C-13 was esterified by the octanoic acid moiety.

In the NOESY spectrum of **1**, a series of correlation signals such as H-8/H-11, H-8/H-17, H-8/OH-4, H-7/H-14 and H-10/OH-9 were observed. Acetylation of **1** with acetic anhydride and pyridine (1:1) at room temperature gave **1a**. In the ^{1}H NMR spectrum of **1a**, the two proton signals of the methylene group at C-5 exhibited signals at δ 2.38 and 2.52 ppm. These shifts and according to literature [3], the OH-4 should be in β configuration as shown in Fig. 1. The ^{13}C NMR data of the diterpenic skeleton of **1** were also found identical to those of a newly reported compound 12-deoxyphorbol-13-hexadecanoate [4], therefore, all the chiral centers in **1** should possess the same configurations as in 12-deoxyphorbol. Compound **1** was thus determined to be 12-deoxyphorbol-13-octanoate. It is a new natural product and has been named mellerin A.

Compound **2** was obtained as a colourless gum. Negative ion mode D/CI mass spectrum of **2** gave a

quasimolecular ion peak at 503 $[M-H]^-$, while its EI mass spectrum exhibited a molecular ion peak at *m/z* 504 $[M]^{+\cdot}$, which indicated its molecular weight to be 504. In the ^1H NMR spectrum of **2**, four methyl signals were observed at δ 0.88 (*t*, 6.0), 1.47 (*d*, 6.0), 1.80 (*br s*) and 1.92 (*br s*). One olefinic proton signal was found at δ 7.59 (*br s*). According to the ^{13}C NMR and DEPT spectra of **2**, 28 carbon signals were differentiated as four methyls, nine methylenes, seven methines and eight quaternary carbon signals. The ^1H and ^{13}C NMR data of **2** were similar to those of **1**. Furthermore, the UV spectrum of **2** exhibited a maximum at λ 241 nm. Therefore, **2** was an analogue of **1**. The ^{13}C NMR data of **2** revealed the existence of two quaternary carbon signals at δ 119.2 and 146.2 ppm and one methylene at δ 111.0 ppm. No carbonyl carbon signal belonging to a typical ester group was found as in the ^{13}C NMR spectrum of **1**. The quaternary carbon signal at δ 111.0 ppm suggested the existence of an ortho ester function as found in some characteristic compounds from Euphorbiaceae and Thymelaeaceae plants [5, 6]. Analysis of DQF-COSY, TOCSY, HMQC and HMBC spectra of **2** led to the establishment of its structure and assignment of all proton and carbon signals (Fig. 1, Table 1). Compound **2** possessed a daphnane-type skeleton.

In the NOESY spectrum of **2**, main correlation signals were observed at H-5/H-10, H-7/H-8, H-7/H-14, H-8/H-11, H-8/H-12β, H-8/H-14, H-11/H-12β and H-12α/H-18. Furthermore, the ^{13}C NMR data of the diterpenic moiety of **2** were found identical to those of huratoxin [5]. Therefore, **2** and huratoxin should possess an identical diterpenic skeleton. The ^{13}C NMR data of **2** were assigned according to the results of various 2D experiments (Table 1). The ^{13}C NMR data (C-4, C-5, C-7, C-9, C-10, C-13, C-14, C-17, C-18 and C-19) belonging to the skeleton of huratoxin were not exactly assigned in the literature due to no 2D NMR spectra were available in the past [5]. To our best knowledge, compound **2** is a new natural product and named mellerin B.

The skeletons of **1** and **2** are well known in some compounds isolated from Euphorbiaceae and Thymelaeaceae species [4, 5]. Diterpenes **1**, **2** and other reported compounds only differed in the carbon chain lengths.

Compounds **3**–**5** were also isolated from the dichloromethane extract. Their structures were identified by spectroscopic methods and also by comparison with data reported in the literature as (24*R*)-24-ethylcholesta-3β,5α,6β-triol (**3**) (also from the sponge *Cliona copiosa* of the family Cliondae) [7], 7β-hydroxysitosterol (**4**) (also from the aquatic plant *Typha latifolia* of the family Typhaceae and the sponge *Corallistes undulatus* of the order Lithistida) [8, 9] and β-sitosterol (**5**).

During the powdering of the plant material and isolation of compounds **1** and **2**, pungent feeling in the nose were noticed. It is well known that many similar diterpenes from Euphorbiaceae plants possess such properties. Therefore, the two diterpenes **1** and **2** were probably responsible for this effect.

Phytochemical investigation of the polar components of *N. melleri* are being undertaken.

EXPERIMENTAL

General

Melting points were measured with a Mettler-FP-80/82 hot stage apparatus and uncorrected. UV spectra were measured using a Varian DMS 100S UV Visible spectrophotometer. $[\alpha]_D$ were measured with a Perkin–Elmer 241 MC polarimeter. NMR spectra were obtained on Varian VXR 200 and Varian Innova 500 MHz instruments. Chemical shifts were reported in δ (ppm) with residual $CHCl_3$ signals (7.26/77.0) as internal standards. D/CI and EI mass spectra were recorded on a Finnigan MAT TSQ 700 instrument. NH_3 was used in the case of D/CI experiments.

Plant material

The leaves of *Neoboutonia melleri* were collected at Christon Bank, Mazowe, Zimbabwe. Voucher specimens have been deposited at the National Herbarium, Harare, Zimbabwe and at the Institut de Pharmacognosie et Phytochimie in Lausanne (No. 96132).

Extraction and isolation

Dried leaves of *Neoboutonia melleri* (1.6 kg) were ground under liquid nitrogen. The ground plant material was then extracted with CH_2Cl_2 and MeOH successively at room temperature (4 × 10 l). The filtrates were then evaporated to dryness under 40° *in vacuo* to give a CH_2Cl_2 extract (64 g) and a MeOH extract (152 g), respectively.

The CH_2Cl_2 extract (60 g) was subjected to chromatography on silica gel (230–400 mesh) column, with a Et_2O–Me_2CO gradient (4:1 → 0:1) and MeOH, successively, to give seven frs (I–VII). Compound **5** (500 mg) was crystallised from fr. II. The residue of fr. II was filtered through a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) and then separated on a RP-18 Lobar column, with a mixture of MeOH and H_2O (9:1) to give finally compound **1** (150 mg). Fr. III (3.2 g) was subjected to Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) and then chromatographed on silica gel column with $CHCl_3$–Me_2CO (5:1) followed by prep. TLC with a mixture of Et_2O–Me_2CO (3:2) as developing solvent to give compound **2** (200 mg). Fr. V was subjected to silica gel column with a $CHCl_3$–Me_2CO gradient (2:1 → 1:1), and then to Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) to give compounds **3** (8 mg) and **4** (10 mg).

Compound **1**. Colourless gum. $[\alpha]_D^{25}$ +40.3° (Me_2CO, *c* 5.3). UV λ_{max} MeOH nm (log ε): 235 (3.74). Positive ion mode D/CI-MS: *m/z* 492 $[M+NH_4]^+$ and 475 $[M+H]^+$, negative ion mode D/CI-MS: *m/z* 473

Table 1. ^1H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR data of **1** and **2**

	1		**2**	
No.	^1H	^{13}C	^1H	^{13}C
1	7.56, *s*	161.2, *d*	7.59, *s*	161.1, *d*
2		132.7, *s*		136.4, *s*
3		209.5, *s*		209.7, *s*
4		73.7, *s*		72.4, *s*
5α	2.45, *d*, 19.0	38.3, *t*	4.24, *br s*	71.4, *d*
5β	2.55, *d*, 19.0			
6		140.1, *s*		60.6, *s*
7	5.65, *d*, 5.0	130.1, *d*	3.42, *s*	64.2, *d*
8	3.00, *s*	38.9, *d*	2.88, *d*, 2.5	36.5, *d*
9		76.2, *s*		78.6, *s*
10	3.20, *s*	55.5, *d*	3.73, *br s*	48.1, *d*
11	1.96, *m*	36.1, *d*	2.44, *m*	34.7, *d*
12α	1.53, *m*	31.8, *t*	1.63, *m*	36.3, *t*
12β	2.05, *m*		2.19, *m*	
13		63.3, *s*		84.0, *s*
14	0.80, *d*, 6.0	32.4, *d*	4.36, *d*, 2.5	81.6, *d*
15		22.6, *s*		146.2, *s*
16a	1.18, *s*	23.2, *q*	4.88, *br s*	111.0, *t*
16b			5.00, *br s*	
17	1.07, *s*	15.3, *q*	1.76, *br s*	18.9, *q*
18	0.85, *m*	18.5, *q*	1.14, *d*, 7.0	20.2, *q*
19	1.70, *br s*	10.0, *q*	1.78, *br s*	9.8, *q*
20a	3.92, *d*, 13.0	68.1, *t*	3.78, *d*, 12.5	65.2, *t*
20b	4.00, *d*, 13.0		3.83, *d*, 12.5	
1′		175.9, *s*		119.2, *s*
2′	2.26, *m*	34.5, *t*	1.92, *m*	34.8, *t*
3′	1.60, *m*	24.7, *t*		23.4, *t*
4′	1.30, *m*	29.0, *t*		
5′	1.25, *m*	28.8, *t*		
6′	1.25, *m*	31.5, *t*		31.8, *t*
7′	1.28, *m*	22.5, *t*		22.6, *t*
8′	0.86, *t*, 7.0	14.0, *q*	0.87, *t*, 7.0	14.0, *q*

$[M-H]^-$. ^1H and ^{13}C NMR ($CDCl_3$) data: shown in Table 1. ^1H NMR (d_6-DMSO, 500 MHz, 35°C) data δ: 7.46 (1H, *s*, H-1), 5.57 (1H, *s*, OH-4), 5.47 (1H, *d*, 5.0, H-7), 4.67 (1H, *s*, OH-9), 4.66 (1H, *t*, 5.5, OH-20), 3.76 (2H, *br s*, H-20), 2.97 (1H, *s*, H-10), 2.83 (1H, *s*, H-8), 2.35 (1H, *d*, 19.0, H-5β), 1.65 (3H, *br s*, H-19), 1.05 (3H, *s*, H-16), 0.96 (3H, *s*, H-17), 0.84 (3H, *t*, 7.0, H-8′), 0.79 (3H, *d*, 7.0, H-18), 0.77 (1H, *d*, 6.0, H-14). ^{13}C NMR (d_6-DMSO, 125 MHz) data δ: 208.3(*s*, C-3), 174.5(*s*, C-1′), 159.2(*d*, C-1), 140.7 (*s*, C-6), 132.4 (*s*, C-2), 127.8 (*d*, C-7), 74.9 (*s*, C-9), 73.0 (*s*, C-4), 66.1 (*t*, C-20), 63.4 (*s*, C-13), 56.1 (*d*, C-10), 38.1 (*d*, C-8), 37.2 (*t*, C-5), 35.8 (*d*, C-11), 33.6 (*t*, C-2′), 32.3 (*d*, C-14), 31.3 (*t*, C-12), 31.1(*t*, C-6′), 28.4 (*t*, C-4′), 28.3 (*t*, C-5′), 24.4 (*t*, C-3′), 22.9 (*q*, C-16), 22.7 (*s*, C-15), 22.0 (*t*, C-7′), 18.6 (*q*, C-18), 15.5 (*q*, C-17), 13.9 (*q*, C-8′), 10.0 (*q*, C-19).

Acetylation of **1** Compound **1** (15 mg) was dissolved in 5 ml of a mixture of acetic anhydride and pyridine (1:1). After heating at 60° for 2 h, the solvent was evaporated to dryness *in vacuo* and the residue was purified on silica gel column with *n*-hexane–Me_2CO (3:1) as eluent to give **1a** (15 mg).

Compound **1a**. Colourless oil. $[\alpha]_D^{25}$ +49.6° (Me_2CO, *c* 1.0). Negative ion mode D/CI-MS: *m/z* 515 $[M-H]^-$. ^1H (500 MHz, $CDCl_3$) data δ: 7.60 (1H, *s*, H-1), 5.72 (1H, *d*, 4.5, H-7), 4.47 (1H, *d*, 12.5, H-20a), 4.44 (1H, *d*, 12.5, H-20b), 3.29 (1H, *br s*, H-10), 3.00 (1H, *br s*, H-8), 2.52 (1H, *d*, 19.0, H-5β), 2.38 (1H, *d*, 19.0, H-5α), 2.05 (3H, *s*, CH_3CO), 1.78 (3H, *br s*, H-19), 1.19 (3H, *s*, H-16), 1.06(3H, *s*, H-17), 0.88 (6H, *m*, H-18 and H-8′), 0.81 (1H, *d*, 5.5, H-14).

Compound **2**. Colourless gum. $[\alpha]_D^{25}$ +48.9° (Me_2CO, *c* 8.2). UV λ_{max} MeOH nm (log ε): 241 (3.95). Negative ion mode D/CI-MS: *m/z* 503 $[M-H]^-$, EI-MS: *m/z* 504 $[M]^{+\cdot}$. ^1H and ^{13}C NMR ($CDCl_3$) data: shown in Table 1.

Acknowledgement—The authors would like to thank the Swiss National Science Foundation for financial support of this work.

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