ORGANIC LETTERS

2013
Vol. 15, No. 7
1580–1583

Norfriedelins A–C with Acetylcholinesterase Inhibitory Activity from Acerola Tree (*Malpighia emarginata*)

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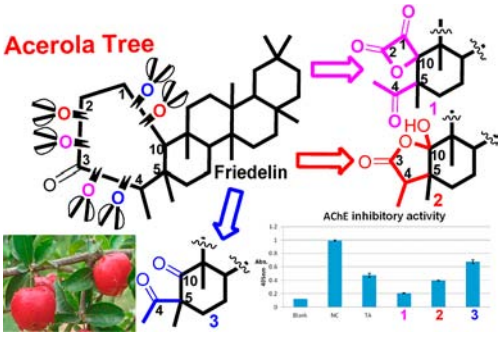
Received February 6, 2013

ABSTRACT



Three novel norfriedelanes, A–C (1–3), were isolated from the branches and roots of *Malpighia emarginata*. Their structures and absolute configurations were determined by 1D and 2D NMR techniques and X-ray crystallographic analysis. Norfriedelin A (possessing an α-oxo-β-lactone group) and norfriedelin B (with a keto-lactone group) showed acetylcholinesterase inhibitory effects with the IC_{50} values of 10.3 and 28.7 μM, respectively.

Acerola (*Malpighia emarginata* DC) comprises 30 species of shrubs native to the West Indies. It belongs to the Malpighiaceae family. Until recently, the plant has been known by the synonymous *M. glabra* and *M. punicifolia*, but taxonomic work has resulted in the acceptance of *M. emarginata* as the current scientific name for this plant.[1] In China, *M. emarginata* was cultivated popularly in the south, such as GuangXi and GuangDong Provinces, since its fruit is recognized as a functional food due to its high contents of vitamin C.[2] Previous investigations mainly focused on the constituents and bioactivities of its fruits[3] but seldom on those of its whole plants (leaves, flowers, blanches, and roots).

Friedelanes, a kind of pentacyclic triterpene, showed extensive bioactivities such as insulin sensitization effects, cytotoxicity, aldose reductase inhibition, and antinociceptive activity.[4] Although natural or synthetic A-seco-friedelanes were reported,[5] natural norfriedelane triterpenoids in ring A have been seldom reported, except for some synthetic products.[6]

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(1) Howard, R. A. *Flora of the Lesser Antilles Dicotyledonae*; Arnold Arboretum, Jamaica Plain: Boston, MA, 1988; Vol. 4, part 1.

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10.1021/ol4003702
Published on Web 03/13/2013

Our chemical investigation of the branches and roots of *M. emarginata* resulted in the isolation of three unusual ring A norfriedelanes, A–C (**1**–**3**) (Figure 1). Norfriedelin A (**1**) is a 3-norfriedelane, possessing the α-oxo-*β*-lactone group, and norfriedelin B (**2**) is a 1,2-dinorfriedelane with a keto-lactone group, while norfriedelin C (**3**) is a 1,2,3-trinorfriedelane. In this communication, the isolation and structural elucidation of compounds **1**–**3** are described, as well as their acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities and a plausible biosynthetic route.

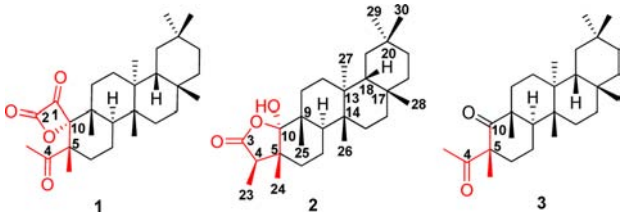

Figure 1. Structures of norfriedelins A–C (**1**–**3**).

Compound **1**[7] was obtained as optically active colorless needle crystals ($[\alpha]_D^{21}$ −12.3). The molecular formula $C_{29}H_{44}O_4$ was established from the positive-ion HREIMS at *m*/*z* 456.3238 $[M]^+$ (calcd 456.3240). The IR spectrum showed absorptions at 1839, 1763, and 1690 cm^{-1}, revealing the existence of *β*-propiolactone and carbonyl groups. In the ^1H NMR spectrum of **1**, 8 singlet methyl signals were observed occurring at δ_H 0.94, 0.98, 1.03 (6H, 2 × CH_3, each s), 1.16, 1.27, 1.37, 2.13 (Table 1). The ^{13}C NMR spectrum gave 29 carbon resonances, which were sorted into 8 methyls, 9 methylenes, 2 methines, and 10 quaternary carbons (Table 2). Moreover, several important functionalities, including two ketone carbonyls (δ_C 191.3 s, 211.9 s), one conjugated ester carbonyl (δ_C 164.1 s), and one oxygenated quaternary carbon (δ_C 101.6 s), were also identified. Taking into consideration 29 carbon atoms, 8 degrees of unsaturation, in combination with the number of methyls, compound **1** may be formulated of a seco-A-ring norfriedelane. The ^{13}C NMR data of the B, C, D, and E rings were similar to those in friedelin and friedelinol.[8]

All of the B, C, D, and E rings' carbon signals were assigned on the basis of careful analysis of the HMBC, ^1H–^1H COSY, and HSQC data for **1** (Figure 2a).

In the HMBC spectrum, the oxygenated quaternary carbon signal at δ_C 101.6 (s) correlated with two methyl signals at δ_H 1.37 (Me-24) and δ_H 1.27 (Me-25); meanwhile, the conjugated ketone carbonyl (δ_C 191.3 s) and ester carbonyl (δ_C 164.1 s) showed no correlations with other signals. The 1-keto-2,10-lactone group was deduced from the above data together with the IR absorption at 1839 cm^{-1}. The ketone carbonyl group at C-4 was established by the HMBC correlations of Me-23 (δ_H 2.13) with C-4 (δ_C 211.9) and C-5. The ROESY correlations of Me-24/Me-25, Me-25/Me-26, Me-28/Me-30 and H-18, and Me-27/H-8 indicated that their relative configurations were the same as friedelane-type triterpenes such as friedelin and friedelinol (Figure 2b).

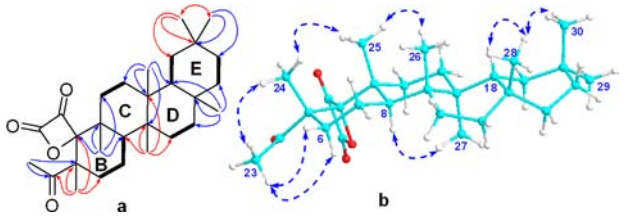

Figure 2. ^1H–^1H COSY (—) and selected HMBC (→) correlations of **1** (a). Selected ROESY correlations (←-→) of **1** (b).

However, the absolute configuration of C-10 could not be deduced by the ROESY experiment. X-ray diffraction analysis of **1**[9] with Cu Kα radiation, which resulted in a Flack parameter of 0.09(15), did confirm the proposed structure and also allowed unambiguous assignment of the absolute configuration of **1** as drawn (Figure 3). Finally, the structure of **1** was deduced as 1,4-dioxo-2,10-lactone 3-norfriedelin and named as norfriedelin A.

Norfriedelin B (**2**)[10] was obtained as colorless crystals (in $CHCl_3$) and displayed a molecular ion $[M]^+$ at *m*/*z*

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(7) Norfriedelin A (**1**): mp 350–353 °C; colorless needle crystals; $[\alpha]_D^{21}$ −12.3 (*c* 0.30, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.16) nm; IR (KBr) ν_{max} 2927, 2867, 1839, 1763, 1690, 1465, 1145, 1244, 1117, 1037, 826 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m*/*z* 479 $[M + Na]^+$; positive HREIMS *m*/*z* 456.3238 $[M]^+$ (calcd for $C_{29}H_{44}O_4$ $[M]^+$, 456.3240).

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(9) X-ray data of **1**: $C_{29}H_{44}O_4$, *M* = 456.64, monoclinic, *a* = 6.42080(10) Å, *b* = 12.4697(2) Å, *c* = 15.6648(2) Å, α = 90.00°, *β* = 93.3890(10)°, *γ* = 90.00°, *V* = 1252.02(3) Å^3, *T* = 100(2) K, space group *P*21, *Z* = 2, *μ*(Cu Kα) = 0.615 mm^{-1}, 10383 reflections measured, 3969 independent reflections (R_{int} = 0.0319). The final R_1 values were 0.0352 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0913 ($I > 2\sigma(I)$). The final R_1 values were 0.0352 (all data). The final $wR(F^2)$ values were 0.0914 (all data). The goodness of fit on F^2 was 1.051. Flack parameter = 0.09(15). The Hooft parameter is 0.03(5) for 1665 Bijvoet pairs. The crystal structure of **1** was solved by direct method SHELXS-97 (Sheldrick, G. M. University of Gottingen: Gottingen, Germany, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 910228). Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U.K.; fax (+44) 1223–336–033; or deposit@ccdc.cam.ac.uk).

(10) Norfriedelin B (**2**): mp 231–233 °C; colorless crystals; $[\alpha]_D^{21}$ +1.5 (*c* 0.20, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 212 (2.53) nm; IR (KBr) ν_{max} 3428, 2950, 2927, 2867, 1690, 1460, 1386, 1283, 1208, 976 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m*/*z* 453 $[M + Na]^+$; positive HREIMS *m*/*z* 430.3430 $[M]^+$ (calcd for $C_{28}H_{46}O_3$ $[M]^+$, 430.3447).

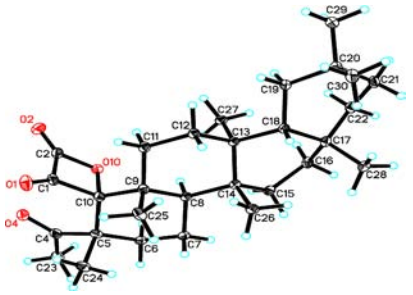

Figure 3. Single-crystal X-ray diffraction of norfriedelin A (**1**).

Table 1. ^{1}H NMR Data of **1**–**3** in $CDCl_3$ (J in Hz)

no.	**1**[a]	**2**[a]	**3**[b]
4		2.16 (q, 7.6)	
6	1.98 (m), 1.98 (m)	1.69 (m), 1.24 (m)	2.28 (m), 1.50 (m)
7	1.67 (m), 1.60 (m)	1.48 (m), 1.41 (m)	1.76 (m), 1.56 (m)
8	1.72 (m)	1.77 (m)	1.56 (m)
11	1.89 (m), 0.95 (m)	1.99 (m), 1.15 (m)	1.33 (m), 1.27 (m)
12	1.36 (m), 1.36 (m)	1.41 (m), 1.34 (m)	1.60 (m), 1.49 (m)
15	1.50 (m), 1.33 (m)	1.47 (m), 1.27 (m)	1.39 (m), 1.24 (m)
16	1.56 (m), 1.38 (m)	1.55 (m), 1.36 (m)	1.44 (m), 1.29 (m)
18	1.53 (m)	1.52 (m)	1.49 (m)
19	1.35 (m), 1.18 (m)	1.38 (m), 1.21 (m)	1.26 (m), 1.16 (m)
21	1.44 (m), 1.28 (m)	1.44 (m), 1.27 (m)	1.37 (m), 1.19 (m)
22	1.51 (m), 0.94 (m)	1.52 (m), 0.93 (m)	1.43 (m), 0.86 (m)
23	2.13 (s)	1.37 (d, 7.6)	2.06 (s)
24	1.37 (s)	1.13 (s)	1.17 (s)
25	1.27 (s)	1.11 (s)	1.05 (s)
26	1.03 (s)	1.01 (s)	1.03 (s)
27	1.03 (s)	1.04 (s)	0.82 (s)
28	1.16 (s)	1.16 (s)	1.08 (s)
29	0.98 (s)	0.98 (s)	0.91 (s)
30	0.94 (s)	0.94 (s)	0.87 (s)

[a] Determined at 600 MHz. [b] Determined at 500 MHz.

430.3430 (calcd 430.3447) in the positive HREIMS analysis, consistent with a molecular formula of $C_{28}H_{46}O_3$ incorporating 6 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3428 cm^{-1}) and carbonyl (1690 cm^{-1}) groups.

The ^{13}C NMR data of norfriedelin B (**2**) (Tables 1 and 2), with the aid of HSQC experiments, revealed one doublet methyl (δ_C 14.1; δ_H 1.37, d, J = 7.6 Hz), seven tertiary methyls (δ_C 17.1, 18.2, 18.4, 19.9, 32.0, 32.1, and 34.7), nine methylenes, three methines, six sp^3 quaternary carbons, one sp^3 oxygenated quaternary carbon (δ_C 112.5), and one ester carbonyl carbon (δ_C 180.8). In addition, the hydroxy proton (δ_H 2.83) was distinguished by HSQC data which were supportive of a hydroxyl group, which was in agreement with the IR spectrum. The above information, especially the data of one doublet methyl and seven tertiary methyls, combined with the molecular ($C_{28}H_{46}O_3$) suggested that compound **2** could be a dinorfriedelane-type triterpenoid.

Table 2. ^{13}C NMR Data of **1**–**3** in $CDCl_3$

no.	**1**[a]	**2**[a]	**3**[b]	no.	**1**[a]	**2**[a]	**3**[b]
1	191.3 s			16	35.9 t	35.8 t	35.5 t
2	164.1 s			17	30.2 s	30.0 s	30.0 s
3		180.8 s		18	42.9 d	42.7 d	43.0 d
4	211.9 s	51.0 d	206.3 s	19	35.3 t	35.1 t	35.1 t
5	59.4 s	43.8 s	61.2 s	20	28.4 s	28.2 s	28.1 s
6	33.0 t	40.4 t	28.9 t	21	32.9 t	32.8 t	32.8 t
7	17.2 t	17.6 t	16.4 t	22	39.2 t	39.0 t	38.8 t
8	44.7 d	44.3 d	42.9 d	23	24.7 q	14.1 q	25.9 q
9	42.0 s	41.9 s	47.2 s	24	21.0 q	17.1 q	22.9 q
10	101.6 s	112.5 s	218.2 s	25	19.2 q	18.2 q	21.6 q
11	28.3 t	26.0 t	29.7 t	26	20.0 q	19.9 q	19.7 q
12	29.4 t	29.4 t	30.1 t	27	18.6 q	18.4 q	18.1 q
13	39.8 s	39.5 s	39.3 s	28	32.3 q	32.1 q	31.8 q
14	38.5 s	37.9 s	39.1 s	29	32.2 q	32. 0 q	32.1 q
15	32.3 t	32.3 t	31.4 t	30	34.9 q	34.7 q	34.5 q

[a] Determined at 150 MHz. [b] Determined at 125 MHz.

Careful comparison of the NMR data of **1** and **2** (Tables 1 and 2) showed that the B, C, D, and E ring signals were very similar. All of the B, C, D, and E rings' hydrogen and carbon signals of **2** were identified by the HMQC, ^{1}H–^{1}H COSY, and HMBC spectra (see the Supporting Information).

The HMBC correlations from Me-23 (δ_H 1.37) to C-3 (δ_C 180.8), C-4, and C-5; from Me-24 (δ_H 1.13) to C-4, C-5, C-6, and C-10 (δ_C 112.5); from Me-25 (δ_H 1.11) to C-10, C-9, C-8, and C-11; from 10-OH (δ_H 2.83) to C-10 and C-5, along with the ^{1}H–^{1}H COSY correlations of H-4/Me-23, established the 10-hydroxy-3,10-lactone fragment (ring A) (Figure 4). The relative configuration of 10-OH was arbitrarily assigned as α-oriented by the ROESY correlation of 10-OH/H-4α.

Norfriedelin C (**3**)[11] was obtained as colorless crystals (from $CHCl_3$), mp 210–212 °C. The molecular formula was determined as $C_{27}H_{44}O_2$ by positive-ion HRESIMS in conjunction with NMR data. The IR spectrum showed a carbonyl group (1692 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed singlets for eight tertiary methyl groups at δ_H 0.82, 0.87, 0.91, 1.03, 1.05, 1.08, 1.17, and 2.06. The ^{13}C NMR spectrum of **3** displayed 27 carbon resonances, which were assigned by HSQC experiments as 8 methyls, 9 methylenes, 2 methines, and 8 quaternary carbons (including two carbonyl signals at δ_C 206.3 and 218.2). It could be a trinorfriedelane-type triterpenoid from the data above.

The ^{13}C NMR data for compound **3** (Table 2) were closely related to those of **1** except for the absence of the characteristic resonances for the β-propiolactone group, as in **1**, and the presence of one lower-field carbon signal at δ_C 218.2. In the HMBC spectrum (Figure 4), the signal at δ_C 218.2 correlating with Me-24, Me-25, H-11, and H-6 indicated that the location of the carbonyl group was at

(11) Norfriedelin C (**3**): mp 210–212 °C; colorless crystals; $[\alpha]_D^{21}$ +10.3 (c 0.34, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (2.57) nm; IR (KBr) ν_{max} 2951, 2926, 2868, 1692, 1462, 1387, 1359, 1142, 1052, 1015, 977 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS m/z 423 $[M + Na]^+$, m/z 439 $[M + K]^+$; positive HREIMS m/z 400.3328 $[M]^+$ (calcd for $C_{27}H_{44}O_2$ $[M]^+$, 400.3341).

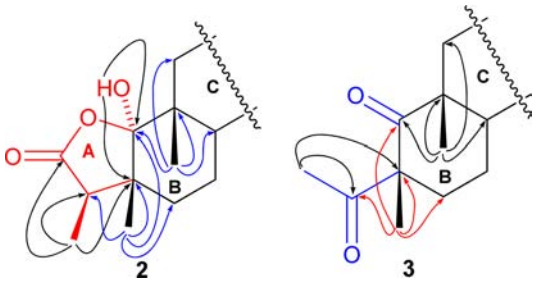

Figure 4. Key HMBC (→) correlations of **2** and **3**.

C-10. The HMBC correlations of Me-23/C-4 (δ_C 206.3) and C-5, Me-24/C-4, C-5, C-6, and C-10 further determined the two carbonyl groups at C-4 and C-10, respectively.

Plausible biogenetic pathways for **1**−**3** have been proposed in Scheme 1. The new norfriedelane might be derived from the natural friedelin (**4**), which was also isolated from this plant. Friedelin would be translated to cerin, calotropfriedelenol, or friedelane-1,3-dione by oxidation or reduction since friedelin and three of them could be present in the plants.[12] Further oxidation, dehydration, or oxidative decarboxylation could afford compounds **1**−**3**, respectively.

Hypothetically, inhibitors of AChE could increase the efficiency of cholinergic transmissions by preventing from the hydrolysis of released acetylcholine (Ach), which has been reported to be associated with the onset of Alzheimer's disease (AD).[13] Therefore, enhancement of ACh levels by using potent AChE inhibitors in the brain has been considered to be an effective approach for treating AD.[14]

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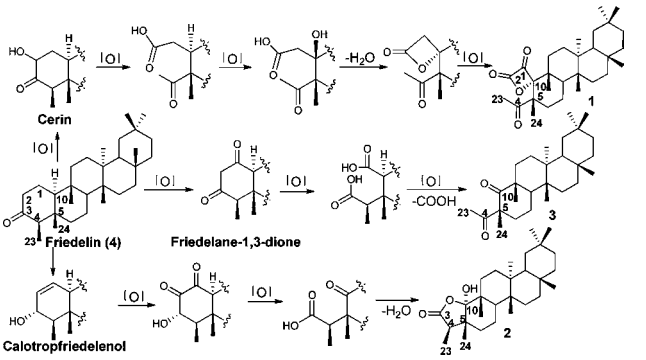
Scheme 1. Plausible Biogenetic Routs of **1**−**3**



The AChE and BuChE inhibitory activities of compounds **1**−**3** were assayed using the Ellman method (details in Supporting Information).[15] Compounds **1** and **2** showed AChE inhibitory activities with IC_{50} values of 10.3 and 28.7 μM, respectively, and compound **2** also showed weak BuChE inhibitory activity (% inhibition was 33.7% at a concentration of 50 μM). Tacrine was used as the positive control (AChE inhibition IC_{50} 0.26 μM; BuChE inhibition percentage was 52% at a concentration of 0.33 μM). Compound **3** was inactive at 50 μM.

Acknowledgment. This project was supported by Amway R&D project (R&D20111027Z), NSFC (No. 81202437), the High-Tech Special Project of Yunnan Province (0703C51211), and CAS (Y0225211Y1), the Major Drug Discovery Project of MOST (2011ZX09307-002-02).

Supporting Information Available. Experimental procedures, 1D and 2D NMR spectra of norfriedelins A−C (**1**−**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.