

Two New Ring A-Rearranged Clerodane Diterpenes, Dunniana Acids A and B, from *Clausena dunniana*

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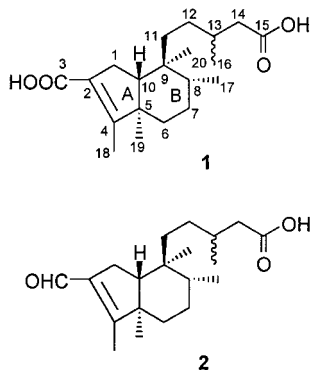
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Two ring A-rearranged clerodane diterpenes named dunniana acids A (**1**) and B (**2**) were isolated from the aerial parts of *Clausena dunniana*. The structures of **1** and **2** were determined using spectral methods.

Clerodane diterpenes are obtained mainly from the plant families Compositae, Euphorbiaceae, and Labiatae and exhibit various structural forms including *trans*-clerodanes and *cis*-clerodanes (5*S*,10*R*- and 5*R*,10*S*-forms, respectively), norterpenes (nor-, dinor-, and tetranor-clerodanes), seco-clerodanes, and rearranged clerodanes. Many clerodanes possess insect antifeedant activity.^{1–3}

In our continuing investigation of *Clausena* species, we now report the isolation and identification of a new type of ring A-rearranged clerodane diterpenes from *Clausena dunniana* Lévl. (Rutaceae), a shrub widely distributed in the south of the People's Republic of China.⁴ These unusual ring A-rearranged diterpenes, dunniana acids A (**1**) and B (**2**), were obtained from the aerial parts of *C. dunniana*, and their structures were elucidated by spectroscopic analysis. Previous studies have revealed that plants of the genus *Clausena* mainly produce carbazole alkaloids^{5–7} and *O*-terpenoidal coumarins.^{8–12} From *C. dunniana*, only three known triterpenoids and some essential oil components have been isolated.^{13,14} There has been no prior report on the isolation of diterpenes from the genus *Clausena*.



Compounds **1** and **2** were separated chromatographically from an EtOAc-soluble extract of *C. dunniana*. Compound **1** was determined to have the molecular formula C₂₀H₃₂O₄ on the basis of high-resolution EIMS, indicating five degrees of unsaturation. The ¹³C NMR data (DEPT) (Table 1) revealed 20 carbon signals, representing five methyl, six methylene, three methine, and six quaternary carbon atoms. The ¹H NMR spectrum (Table 1) of **1** showed signals

for three tertiary alkyl methyl groups (δ 1.98, 0.86, 0.80) and two secondary alkyl methyls (δ 0.96, 0.75). In the HMBC experiment (Table 1), the ¹H–¹³C NMR long-range correlations between the protons on a vinyl methyl group (δ _H 1.98, δ _C 11.7) and carbons at δ _C 50.6 (2 bond), 125.9 (3 bond), and 169.0 (2 bond), and between protons on an alkyl methyl group (δ _H 0.86, δ _C 16.9) and carbons at δ _C 34.4, 50.6, 54.1 (3 bond), and 169.0, afforded partial structure **1a** (Figure 1). In the HMBC experiment (Table 1), the protons on two vicinal methyl groups δ 0.80 (δ _C 18.0) and 0.75 displayed correlations with carbons at δ _C 36.8, 37.3, 37.7, and 54.1, and δ _C 28.4, 36.8, and 37.7, respectively, leading to **1b** (Figure 1). Similarly, **1c** (Figure 1) was generated from the ¹H–¹³C NMR long-range correlations between the secondary alkyl methyl at δ _H 0.96 (δ _C 19.8) and methylene carbons at δ _C 29.4 and 41.4 and the methine carbon at δ 30.8. Moreover, ¹H–¹³C NMR long-range correlations between δ _H 1.59 (δ _C 54.1 in **1a** and **1b**) and δ _C 18.0, 34.4, 37.7, and 169.0 allowed the combination of **1a** and **1b** to afford **1d** (Figure 1), which was further proven by the linear coupling relationship between a methine proton (δ _H 1.59) on the carbon at δ 54.1 and methylene protons (δ 2.27) on the carbon at δ 29.3 in the ¹H–¹H COSY spectrum of **1**. Further, in the ¹H–¹H COSY spectrum, the signal at δ _H 1.29 (δ _C 29.4) showed linear coupling with protons at δ _H 1.18 (δ _C 37.3) and 1.29 (δ _C 37.3), suggesting that δ _C 29.4 was linked with δ _C 37.3 via a carbon–carbon bond, which was further supported by the ¹H–¹³C NMR long-range correlations between δ _H 1.29 (δ _C 29.4) and δ _C 19.8 and 37.3, and between δ _H 1.18 and 1.29 (δ _C 37.3) and δ _C 18.0, 29.4, 36.8, 37.7, and 54.1, permitting fragments **1c** and **1d** to be joined to one another. The location of the carboxylic group at δ _C 179.7 was determined by inspecting the HMBC data (correlation between δ _H 1.88 and δ _C 179.7, and the correlation between δ _H 2.36 and 2.17 and δ _C 179.7), completing the assignment for the residue **1e**. The structure of **1e** is similar to those of the clerodane diterpenes, especially in ring B and the side chain at C-11.^{15–18} In the HMBC spectrum (Table 1), the ¹H–¹³C NMR long-range correlations between the methylene protons appearing at δ _H 2.27 (δ _C 29.3) and the carbon signals at δ _C 50.6, 54.1, 125.9, 169.0, and 172.6 revealed the structure of ring A. This elucidation was consistent with the ¹³C NMR assignment for C-4 (δ _C 169.0 s). Its downfield chemical shift was attributed to the adjacent C-3 carboxyl group. Moreover, compound **1** has a ring A similar to a known triterpene, *tert*-butyl A-norfriedel-2(4)-en-2-carboxylate, especially the ¹³C NMR signals of C-2, C-3, and C-4.¹⁹ NOE correlations between H-8 (δ _H 1.45) and H-10 (δ _H 1.59), and between

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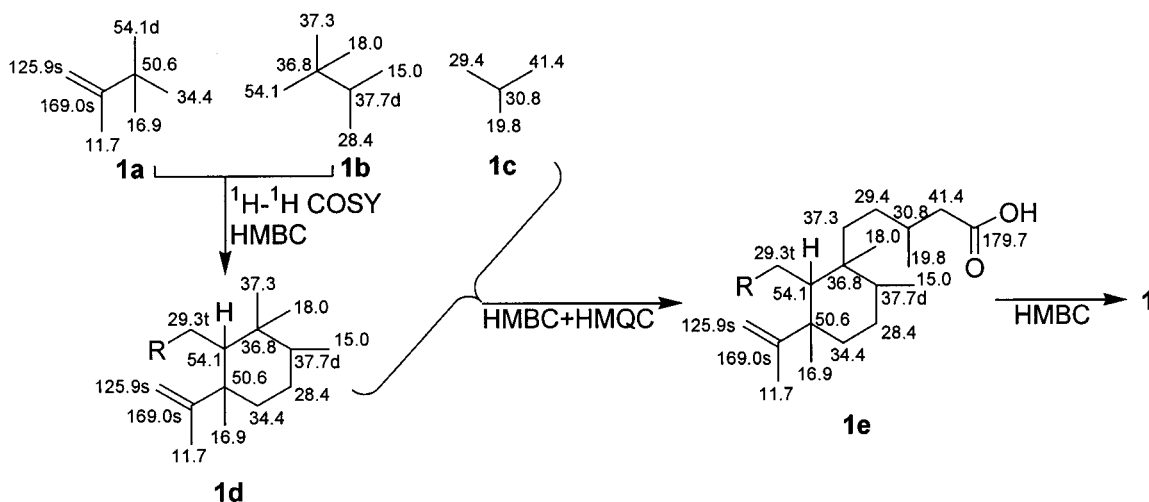
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Table 1. NMR Data for **1** in CDCl₃

position	¹ H ^a	¹³ C	¹ H- ¹ H COSY	HMBC ^b
1	2.27, d (2H, 8.5)	29.3, t	10	2-5, 10
2		125.9, s		
3		172.6, s		
4		169.0, s		
5		50.6, s		
6	1.57, m (2H)	34.4, t	7	5, 7, 8, 10, 19
7	1.48, m (2H)	28.4, t	6	5, 9
8	1.45, m	37.7, d	17	7, 9, 17, 20
9		36.8, s		/
10	1.59, m	54.1, d	1, 11	1, 2, 4, 5, 6, 8/11, 20
11	1.29, m 1.18, m	37.3, t	10-12	8-10, 12, 20
12	1.29, m (2H)	29.4, t	11	11, 16
13	1.88, m	30.8, d	16	11, 12, 14-16
14	2.36, dd (15.0, 6.5), 2.17, m	41.4, t	14	13, 15, 16
15		179.7, s		
16	0.96, d (3H, 6.6)	19.8, q	13	12-14
17	0.75, d (3H, 5.9)	15.0, q	8	7-9
18	1.98, s (3H)	11.7, q		2, 4, 5
19	0.86, s (3H)	16.9, q		4-6, 10
20	0.80, s (3H)	18.0, q		8-11

^a Coupling constants are presented in Hz, and unless otherwise indicated, all proton signals integrate to 1H. ^b Proton showing HMBC correlation to indicated carbon.

**Figure 1.** Structural fragments of **1**.

H-19 (δ_{H} 0.86) and H-20 (δ_{H} 0.80), revealed that rings A and B had a *trans*-junction. A NOE between H-17 (δ_{H} 0.75) and H-20 indicated that H-17 was in α -form. It was not possible to assign the stereochemistry of the C-13 methyl group in this study. Accordingly, **1** (dunniana acid A) was elucidated as 3,4-*seco*-2(4)-cleroden-3,15-dioic acid.

The molecular formula of **2** was assigned by HREIMS (m/z 320.2353) as C₂₀H₃₂O₄. In the ¹H and ¹³C NMR spectra, signals at δ_{H} 9.93 (1H, s) and δ_{C} 188.7 showed the presence of an aldehyde. The ¹H NMR spectrum of **2** exhibited signals for five methyl groups (δ 0.76, 0.80, 0.88, 0.95, and 1.99), the same as for **1**. The ¹³C NMR spectrum of **2** was similar to that of **1** except for the C-3 resonance (Table 1), indicating that **2** has a structure similar to **1**. Differences in the ¹³C NMR spectra between **2** and **1** occurred for C-1, C-2, C-3, and C-4, revealing that the aldehyde group was located at C-3. Therefore, **2**, named dunniana acid B, was elucidated as 3,4-*seco*-3-oxo-2(4)-cleroden-15-oic acid.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Horiba Sepa-300 digital polarimeter. The IR spectra were measured on a Perkin-Elmer-577 spectrophotometer. The NMR spectra were recorded on Bruker AM-

400 and DRX-500 spectrometers. MS were performed on a VG AutoSpec-3000 spectrometer under 70 eV.

Plant Material. The aerial parts of *Clausena dunniana* Lévl. (Rutaceae) were collected at Xishuangbanna, Yunnan, People's Republic of China, in April 1999, and were identified by Prof. D. D. Tao at Kunming Institute of Botany. A voucher specimen (No. H98041703) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, People's Republic of China.

Extraction and Isolation. The powdered aerial parts of *C. dunniana* (2 kg) were extracted with EtOAc (6 L \times 4) under reflux for 6 h each time. The extract (130 g) was separated into five fractions through column chromatography over porous resin D101 (Chemical Factory of Tianjin University, Tianjin, People's Republic of China) by elution with a gradient mixture of ethanol-water from 20% to 100%. Fraction 5 (76.6 g) was subjected to column chromatography over RP-18 eluted with MeOH-H₂O (72:25, 80:20, 100:0). The fraction (55.0 g) obtained by elution with MeOH-H₂O (72:25) was separated into nine portions through column chromatography over Si gel eluted with petroleum ether-EtOAc (90:10, 85:15, 80:20, 70:30, 50:50). The last fraction contained **1** and **2**. Compounds **1** (7 mg) and **2** (15 mg) were further separated through passage over Sephadex LH-20 eluting with Me₂CO.

Dunniana acid A (1): colorless oil; [α]_D²⁵ +281.4° (*c* 0.35, CHCl₃); IR (neat) γ_{max} 3114, 2953, 1697, 1650, 1420, 1381, 1306, 1252, 1218, 1085, 942, 756 cm⁻¹; ¹H and ¹³C NMR data,

see Table 1; EIMS m/z 336 $[M]^+$ (10), 318 (85), 303 (94), 290 (61), 275 (34), 223 (84), 203 (73), 175 (69), 151 (95), 137 (71), 121 (86), 107 (89), 91 (94), 69 (100); HREIMS m/z 336.2310 (calcd for $C_{20}H_{34}O_4$, $[M]^+$ 336.2301).

Dunniana acid B (2): colorless oil; $[\alpha]_D^{23} +83.3^\circ$ (c 0.75, $CHCl_3$); IR (neat) γ_{max} 2948, 2926, 1731, 1697, 1454, 1416, 1385, 1248, 1218, 1108, 766 cm^{-1} ; 1H NMR ($CDCl_3$, 400 MHz) δ 9.93 (1H, s, H-3), 1.99 (3H, s, H-18), 0.95 (3H, d, $J=6.6$ Hz, H-16), 0.88 (3H, s, H-19), 0.80 (1H, s, H-20), 0.76 (3H, d, $J=6.0$ Hz, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 188.7 (d, C-3), 178.6 (s, C-15), 172.0 (s, C-4), 137.2 (s, C-2), 54.1 (d, C-10), 50.8 (s, C-5), 41.4 (t, C-14), 37.5 (s, C-9), 37.3 (t, C-11), 37.2 (d, C-8), 34.1 (t, C-6), 30.8 (d, C-13), 29.8 (t, C-12), 28.4 (t, C-7), 26.2 (t, C-1), 19.8 (q, C-16), 18.1 (q, C-20), 17.1 (q, C-19), 15.2 (q, C-17), 9.6 (q, C-18); EIMS m/z 320 $[M]^+$ (40), $[M - CH_3]^+$ 305 (49), 293 (42), 237 (23), 223 (33), 207 (64), 193 (81), 177 (32), 151 (61), 137 (64), 123 (88), 109 (83), 95 (86), 69 (96), 55 (100); HREIMS m/z 320.2353 (calcd for $C_{20}H_{32}O_3$, $[M]^+$ 320.2351).

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