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Yonarolide A, an unprecedented furanobutenolide-containing norcembranoid derivative formed by photoinduced intramolecular [2+2] cycloaddition

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ABSTRACT

Eight polycyclic furanobutenolide-containing norcembrane diterpenoids featuring C19 frameworks (**1**–**8**) were rapidly recognized and isolated from the Hainan soft coral *Sinularia* sp. by the HSQC-based small molecule accurate recognition technology. Yonarolide A (**1a**), featuring an unprecedented 5/6/4/4/7 pentacyclic ring skeleton, was surprisingly obtained as a transformed product by leaving compound **1** under indoor natural light, and was further proved to be a [2+2] cycloaddition product of **1** by photochemical reaction. The absolute stereochemistry of **1a** and the three known norcembrane diterpenoids **1**, **4**, and **7** were determined by using X-ray diffraction (XRD) analyses. Further, with the aid of XRD analysis, the structure of scabrolide B (**2**), which was previously reported of possessing 5/6/7 tricyclic skeleton, was firmly revised as **2a** with the rare inelegane skeleton featured by the highly oxygenated 5/7/6 tricyclic carbocycle.

Polycyclic furanobutenolide-containing norcembranoids are a rare group of natural products, which are found exclusively in soft corals of the genus *Sinularia*, especially *S. leptoclados, S. inelegans*, and *S. lochmodes* [1–3]. These metabolites have captured the attention of chemists and biologists due to their complex structures featured by a 5/6/7 tricyclic or 5/7/6 tricyclic skeleton, and their wide spread biological activities, such as anti-inflammatory, anti-osteoporotic, and cytotoxic effects [4,5]. Particularly, the first total synthesis of scabrolide A has been accomplished by Stoltz and co-workers in 2020 owning to its potential as an anti-inflammatory agent [6]. However, due to the polycyclic nature and complex stereochemistry of these norditerpenoids, their structure elucidation was challenging, which also limited their further biological study [4,7,8]. In order to explore the chemical diversity of these intriguing furanobutenolide-containing norcembranoids and further confirm their stereochemistry, soft corals from the genus *Sinularia* were collected off the coast of Ximao island and chemically investigated.

Small molecule accurate recognition technology (SMART) is an artificial intelligence-based tool to generate structure hypotheses from HSQC data. It can rapidly recognize the structure types of natural product from crude extracts or fractions of the sample [9]. Gerwick and co-workers used NMR-based SMART for the discovery and rapid classification of several new peptides from a marine cyanobacterium in 2017 [10]. The NMR-based SMART and MS^2-based molecular networking led to the isolation and rapid identification of the macrolide and cyclic peptides in 2020 by Reher *et al.* [11]. This technology is being widely recognized, which inspired us to apply it for the discovery and rapid classification of polycyclic furanobutenolide-containing norcembranoids. The crude extract of species unidentified soft coral *Sinularia* sp. collected off the coast of Ximao Island in the South China Sea, was separated by a rapid silica gel column chromatography to yield five fractions (Fr. A to Fr. E). The cross-peaks obtained from HSQC spectra of crude fractions Fr. A–Fr. E were settled in tables saved as CSV files, which were then uploaded to the SMART system (http://smart.ucsd.edu/classic). The result of Fr. E showed that 5 out of top 8 compounds ordered by cosine similarity score were polycyclic furanobutenolide-containing norcembranoids (Fig. 1). Based on the results of the SMART positioning, Fr. E could be rich in polycyclic furanobutenolide-containing norcembranoids and was thus systematically further investigated for its chemical constituents.

With the help of SMART, eight polycyclic furanobutenolide-containing norcembranoids (**1**–**8**) were rapidly isolated from

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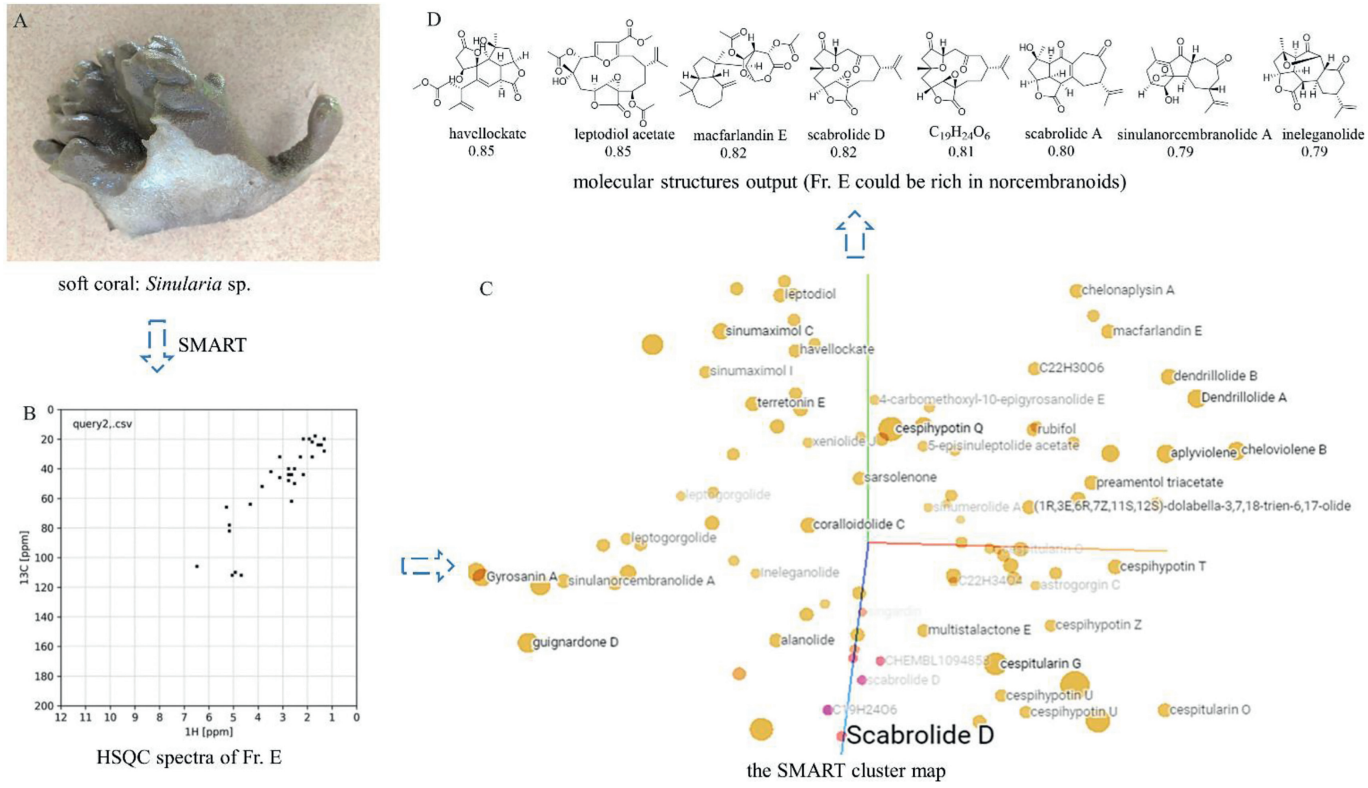


Fig. 1. SMART-guided isolation. (A) Isolated compounds from soft coral *Sinularia* sp.; (B) Digitized HSQC spectrum of Fr. E, which was generated by the SMART system through uploading the files containing the signals in experimental HSQC spectrum of crude extract; (C) The spectra were mapped into a 180-dimensional embedding space (nodes representing natural products trained on the basis of their $^{1}H–^{13}C$-HSQC spectra); (D) SMART 2.0 results (top 8 structures based on cosine similarity score) of Fr. E suggests that it contains norcembranoids.

Sinularia sp. Interestingly, yonarolide A (**1a**), featuring an unprecedented 5/6/4/4/7 pentacyclic carbon skeleton, was obtained as a transformed product by leaving compound **1** in methanol/water under indoor natural light, and further synthesized from **1** by photoreaction. With the aid of X-ray diffraction analysis, the structure of scabrolide B (**2**) was revised as **2a** with the rare inelegane skeleton featured by the highly oxygenated 5/7/6 tricyclic carbocycle. Detailed isolation, structure elucidation of the norcembranoids, and the chemical transformation towards the novel compound **1a** were reported herein.

The acetone extract of the soft coral *Sinularia* sp. was partitioned between Et_2O and *n*-BuOH. The Et_2O-soluble portion was subjected to chromatography to yield five fractions (Fr. A to Fr. E), and the SMART method (Fig. 1, see Supporting information for details) was used to rapidly afford eight pure norcembranoids **1**–**8** (Fig. 2).

Compounds **1** and **3**–**8** were rapidly identified by the comparison of their spectroscopic data with those reported in the literature as yonarolide (**1**) [12], ineleganolide (**3**) [13], scabrolide D (**4**) [14], norcembrene 5 (**5**) [13,15], sinulin D (**6**) [16], 10–epi-gyrosanolide E (**7**) [15], and sinularcasbane O (**8**) [17], respectively. It is worth mentioning that the absolute configurations of compounds **1**, **4** and **7** were unambiguously established for the first time by X-Ray diffraction analyses (Fig. 3).

Interestingly, when we were trying to grow a single crystal for yonarolide (**1**) in a methanol/water 9:1 solvent system, we found that compound **1** has been transformed into **1a** in transparent glass bottle. A semipreparative HPLC (CH_3CN/H_2O, 1:1, v/v) was utilized to analyze the purity of compound **1**. Surprisingly, two principal constituents **1** ($t_R = 7.3$ min) and **1a** ($t_R = 10.6$ min) were observed in HPLC analysis, indicating that compound **1** was transformed into **1a** in the transparent glass bottle under indoor natural light.

The molecular formula of **1a** was established as $C_{19}H_{20}O_4$ with ten double-bond equivalents (DBEs) by ESIHRMS analysis ($[M+H]^+$ *m/z* 313.1437, calcd. for $C_{19}H_{21}O_4$, 313.1434). The 1D NMR and HSQC spectra exhibited 19 carbon resonances, including three carbonyls (δ_C 211.3, 196.8, 175.1), two olefinic carbons (δ_C 127.4, 156.7), four methines (δ_C 80.8, 48.0, 42.1, 34.7), five methylenes (δ_C 43.2, 44.4, 45.3, 29.9, 39.4), two methyl groups (δ_C 16.9, 19.9), three quaternary carbons (δ_C 47.7, 43.3, 47.5). Three carbonyl carbons and two olefinic groups accounted for 4 of the 10 DBEs, the remaining degree of unsaturation required the presence of six rings in **1a**. 1D NMR data comparison of **1** and **1a** suggested that the 5/6/7 tricyclic skeleton and the five-membered lactone ring of **1** was remained. However, the lack of four olefinic carbons (δ_C 149.1, 146.9, 134.1, 111.1) in **1a** suggested that the structural transformation may occur in these carbon positions, probably a [2 + 2] cycloaddition towards two cyclobutane rings. Our hypothesis was further confirmed by HMBC experiment. The clear HMBC correlations from H-17 to C-13/C-15/C-16/C-1 and from H-14 to C-13/C-5/C-15 supported the presence of two cyclobutane rings (Fig. 4), thus constructing a 5/6/4/4/7 pentacyclic ring skeleton in **1a**.

It is obvious that compound **1a** is the photoinduced [2 + 2] cycloaddition product of **1**. To confirm this conversion and to obtain more of **1a** for absolute configuration determination, we carried out the photochemical reaction with different conditions as shown in Table 1. To our delight, a smooth [2 + 2] photocycloaddition between $\Delta^{5,13}$ and $\Delta^{15,16}$ of **1** led to the desired bicyclo[2.2.0]hexane moiety towards **1a** (Table 1, entries 1 and 2). Further, 250 W long arc mercury lamp irradiation either in air or in N_2 proved to be better reaction conditions, with an isolated yield of 48% and 57%,

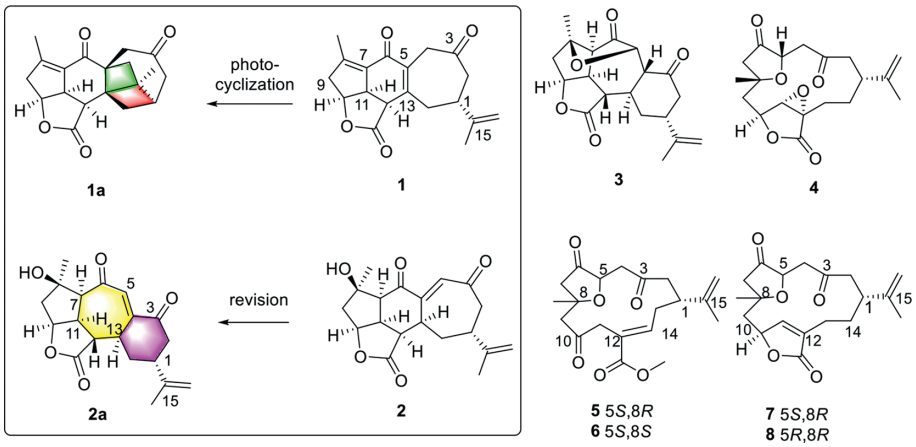


Fig. 2. The structures of compounds **1**–**8**, **1a** and **2a**.

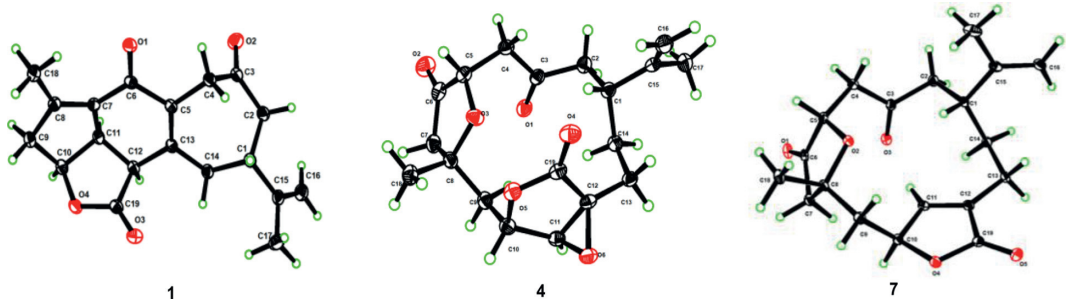


Fig. 3. Perspective ORTEP drawing of X-ray structures of **1**, **4** and **7**.

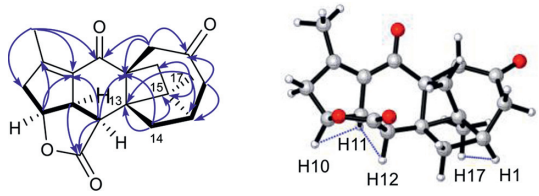
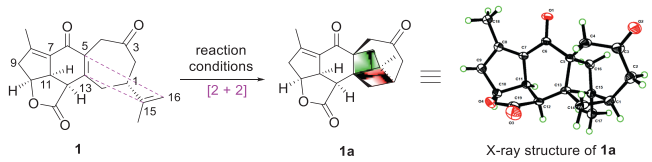


Fig. 4. Key ^{1}H–^{1}H COSY (thick lines), HMBC (blue arrows, from ^{1}H to ^{13}C) and NOESY (blue dashed lines, from ^{1}H to ^{1}H) correlations of compound **1a**.

respectively. Fortunately, after obtaining the enough amount of **1a**, we tried and succeeded in the cultivation of its single crystal, which allowed the unambiguous determination of its absolute configuration and further confirmed its planar structure as 5/6/4/4/7 pentacyclic ring scaffold. It is worth mentioning that the skeleton of **1a** has never been discovered in any known diterpenoid.

Table 1
Screening of the photochemical reaction of **1**.

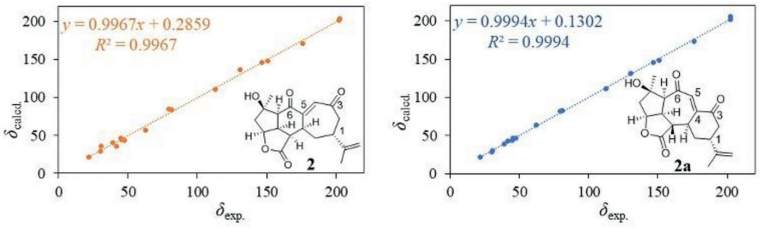


Entry	Reaction conditions[a]	**1a** (%)
1	Indoor natural light, air, 7 d	10[b]
2	Outdoor natural light, air, 7 d	23[b]
3	Dark, air, 7 d	0
4	High-pressure mercury lamp, air, 4 h	48[b], 64[c]
5	High-pressure mercury lamp, N_2, 4 h	57[b], 74[c]

[a] All reactions run in methanol.
[b] Isolated yield.
[c] B.r.s.m. yield.

Compound **2** was readily identified as scabrolide B, a norcembranoid that was previously isolated from the same species but collected off the coast of Kenting, Taiwan, in April 2001 by Sheu and co-workers, based on the overall comparison of its physical and chemical data including NMR spectroscopic data (Table S4 in Supporting information) with those reported in the literature [14]. Since the skeleton of **2** belongs to an extremely rare group of marine natural products, and as far as we known, there are only four members of reported yonarane norditerpenoids, which stimulated our curiosity to double-check the spectroscopic data of **2**. Firstly, we carefully analyzed the data reported in Sheu's paper. They have reported the HMBC correlations from H-4 (δ_H 6.34) to C-5 (δ_C 150.8), C-3 (δ_C 202.2), C-6 (δ_C 202.5), and C-13 (δ_C 41.6), whereas, the correlations from H-5 (δ_H 6.33) to C-7 (δ_C 62.4) and from H-2 (δ_H 2.59) to C-3 (δ_C 202.5), C-15 (δ_C146.4), C-14 (δ_C 30.5), which can be observed in our HMBC spectrum, have not been mentioned by the authors. These cross-peaks are much more consistent with **2a** (C7–C6–C5–C4 linkage), which led to a revision of the planar structure of scabrolide B as possessing a 5/7/6 tricyclic carbocycle.

In order to explain the rationality of the 5/7/6 tricyclic carbocycle and further improve the correctness of our speculation, we calculated the chemical shifts of **2a** and **2** at the PCM/mPW1PW91/6–31+G**//B3LYP/6–31G* level of theory (using chloroform as the solvent) [18,19]. We found the calculated chemical shifts of **2a** to be in significantly better agreement with the experimental values than those calculated for **2** (Table S5 in Supporting information), as indicated by parameters such as correlation coefficient in regression analysis (R^2), mean absolute error (MAE), corrected mean absolute error (CMAE), and maximum absolute error (MaxErr) (Fig. 5). In particular, the remarkably upfield observed chemical



Parameter	2	2a
R^2	0.9967	0.9994
MAE(PPM)	2.60	2.23
CMAE(PPM)	2.60	1.17
MaxErr(PPM)	5.74	3.42
DP4+	0%	100%

Fig. 5. Parameters of the calculated chemical shifts of **2** and **2a**.

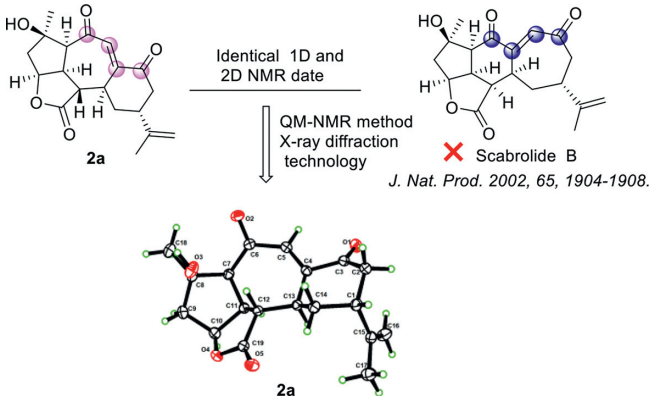


Fig. 6. Revised structure and originally structure of scabrolide B.

shift of C-5 (δ_C 130.5) was much closer to the calculated value in **2a** (δ_C 131.1) than it was to the calculated value in **2** (δ_C 136.6). To address this question with quantifiable confidence, the experimental ^{13}C NMR data were compared to the DFT-NMR data generated from the revised structure of **2a** and the originally elucidated form through DP4+ probability scores [20]. The DP4+ probability scores, resulted in preferring the revised structure (**2a**) over its original version (**2**) with 100% probability (Fig. 5).

Fortunately, the suitable crystal was eventually obtained from CH_2Cl_2-MeOH-H_2O (10:20:1) after many failed attempts and was then sent for X-ray crystallographic analysis using Cu Kα radiation with a Flack parameter of –0.01(8). The X-ray result firmly supported our speculation that the correct structure of scabrolide B was **2a** (Fig. 6). To the best of our knowledge, **2a** is the second compound with the novel inelegane skeleton possessing the highly oxygenated 5/7/6 tricyclic carbocycle in nature [21,22].

In summary, HSQC-based SMART method was applied to guide the isolation of diterpenoids in *Sinularia* sp. collected off the coast of the Ximao Island in the South China Sea, and led to the discovery of eight polycyclic furanobutenolide-containing norcembranoids with C19 frameworks (**1**–**8**). Their structures were elucidated by extensive spectroscopic analysis, NMR calculation with DP4+ probability analysis, and/or X-ray diffraction analyses. Compound **1** belongs to the yonarane norditerpenoids with only four members of this type norditerpenoids. Compounds **2a** and **3** represent the only two examples of inelegane norditerpenoids that have been found in nature. Yonarolide A (**1a**), featuring an unprecedented 5/6/4/4/7 pentacyclic ring skeleton containing a highly strained bicyclo[2.2.0]hexane moiety, was obtained by a chemical transformation from **1**. Compound **1a** is probably a natural product in the soft coral, since many similar [2 + 2] natural product has been found in nature and was confirmed by total synthesis, such as nyingchinoid D and eriobrucinol [23,24]. The discovery of **1**–**8**, especially the novel compound **1a**, has expanded the diversity and complexity of marine diterpenoids. Further study should be conducted for the exploration of the chemistry and biological activities of the novel compound **1a** and its related polycyclic furanobutenolide-containing norcembranoids to understand their roles played in the life cycle of the soft coral.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.05.026.

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