Pure Appl. Chem., Vol. 71, No. 6, pp. 1147–1151, 1999.
Printed in Great Britain.

Search for new compounds and biologically active substances from Chinese marine organisms*

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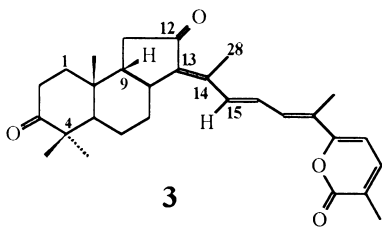
Abstract: In search of new pharmaceutically valuable substances from marine organisms, we have been engaged in chemical studies on the constituents of corals, sponges and algae collected in the South China Sea. This paper summarizes some of our research results. Terpenes, nitrogen-containing compounds and polyhydroxylated sterols are mainly discussed.

The corals, marine sponges and algae are abundant natural resources in the South China Sea. Due to unusual environment as compared with terrestrial organisms, marine organisms produce a variety of substances having various unprecedented chemical structures and exhibiting significant biological activities. In search of new pharmaceutically valuable substances from marine organisms, we have been engaged in chemical studies on the constituents of some 50 species of corals, marine sponges and algae collected in the South China Sea and have found more than 120 new compounds. Many of these exhibited significant biological activities. In this paper, terpenoids, nitrogen-containing compounds and polyhydroxylated sterols are mainly discussed.

Some 50 new terpenoids have been obtained. The most interesting terpenoids are methyl sartortuoate (**1**) and methyl isosartortuoate (**2**). **1** and **2** are tetraterpenes with unique biscembrenoid skeleton. Their structures were determined by spectroscopic methods and X-ray diffraction analysis. Both **1** and **2** exhibited moderate cytotoxicity against P-388 and KB cells. Structurally, compounds **1** and **2** are likely to be formed biogenetically by two cembrenoids via Diels–Alder reaction. We have suggested a biosynthetic path way for these compounds [1,2].

Stelletin A (**3**) was isolated from the sponge *Stelletta tenuis* Linduren (Scheme 1). HRMS *m/z* 463.2874 (M + 1) revealed the molecular formula $C_{30}H_{38}O_4$. The UV spectrum revealed an extended conjugated system like of isomalabaricane-type triterpene (**3A**). Comparison of the ^{1}H NMR data of **3** and



3

Scheme 1

* Invited Lecture presented at the 21st IUPAC International Symposium on The Chemistry of Natural Products (ISCNP-21), Beijing, China, 11–16 October 1998, pp. 1024–1166.
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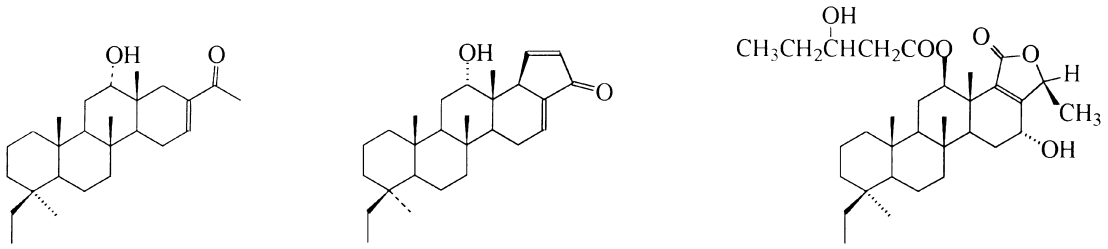
3A revealed the two sets of data to be nearly identical except that the signal for H-28 of **3** was observed at lower field (δ62.35 instead of δ2.07) and that of H-15 was observed at higher field (δ6.99 instead of δ8.26). This indicated that H-28 was deshielded by the C-12 keto group in 3 and so must be *cis*- to it while H-15 is *trans*- to ketone. Thus structure **3** was assigned to this new triterpene (**3**). **3** is strongly toxic toward P388 leukemia cells ($ED_{50} = 1 \times 10^{-3}$ μg/mL) [3].

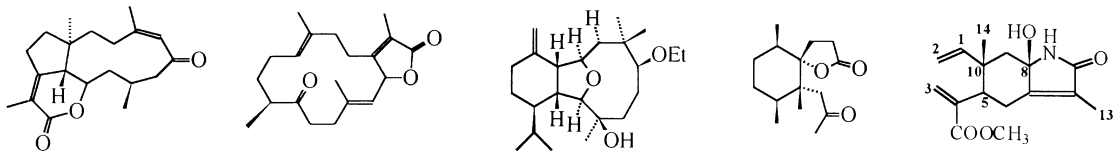
Nineteen new sesterterpenes with bishomosealarane skeleton have been isolated from marine sponge *Phyllosponggia folliascens* [4–6]. Their structures have been determined by elucidation of the spectral data. In comparison to scalarane-type sesterterpenes, an ethyl group at C-4 formed an additional chiral center. The observation of NOE between proton (pro-S) (δ1.53 ppm) and 21-CH_3 protons (δ0.96 ppm) of phyllofenone A (**4**) indicated the β-orientation for ethyl group. A difference NOE technique was helpful for this purpose. Upon saturation the 21-CH_3 (s, 0.68 ppm) clearly showed NOE with the pro-S H-20. The features were identical with that obtained in a 2D J-resolved spectrum. Hence a β-orientation is assioned to the ethyl group [4]. No absolute configuration of any compound of this class was assigned definitely before. We have first established the absolute configuration of this class by using modified Mosher's method. Phyllactone B (**6**), which has a secondary hydroxyl group at C-16, was taken as an example for this purpose. Thus (R)- and (S)-MTPA esters of phyllactone B (**6**) were prepared., proton signals of the corresponding derivatives were assigned by 2D-COSY spectra, respectively. The $\Delta\delta(\delta_s - \delta_R)$ for protons on the E ring were negative, whereas those observed for protons on the B and C rings have positive values. Thus the absolute configuration at C-16 was confirmed as R, and all other chiral centers in this molecule were assigned as 4S, 12R, 24S [5]. Phyllofenone A (**4**) shows anti-fungal activity against *Candida pseudotropicalls* [4]. Phyllofenone B (**5**) [6] showed potent cytotoxicity toward P388 cell line ($IC_{50} = 5$ μg/mL).



4 5 6

Scheme 2

Pathylactone A (**10**) is a rare norsequiterpene with a γ-spirolactone moiety. Its absolute stereochemistry was assigned by CD method [7]. Compound (**9**) exhibited anti-fungal activity [8]. Compounds (**7**) and (**8**) showed potent cytotoxic toward P388 cell lines.



7 8 9 10 11

Scheme 3

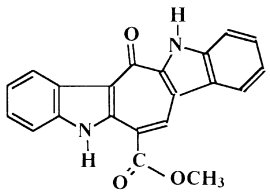
The rare sesquiterpene lactam, clavulinin (**11**), was obtained from the soft coral *Clavularia inflata*. The molecular formula of **11** was established as $C_{16}H_{22}NO_4$ on the basis of FABMS, ^{13}C NMR and DEPT. The existence of one hydroxyl group, two carbonyl groups and three kinds of carbon–carbon double bonds, i.e. CH_2=CH–, CH_2=CR and C=C, were deduced by IR and NMR spectral data. The signals of protons and carbons of the molecule were assigned and connected by ^1H-^1H COSY, ^1H-^{13}C COSY,

HMQC and HMBC techniques. All these data let to establish the structure of clavulanin to be **11**. MS fragmentation fully supported this conclusion. The relative configuration was thus established as 5r*, 8R*, 10S* by interpretation of ^{1}H-^{1}H coupling constants and NOESY data [9].

Different kinds of nitrogen-containing compounds have been isolated from the Chinese marine organisms. The compounds (**12–17**) are selected for discussion in this paper.

Caulersin (**12**), isolated from *Caulerpa serrulata*, is yellowish crystals. **12** gave a M^{+} peak in its HREIMS at *m/z* 32.0992, corresponding to the molecular formula $C_{21}H_{14}N_2O_3$, The ^{13}C NMR signals were similar to those of caulerpin (**12A**). However, the ^{13}C NMR spectrum of **12A** showed only 11 signals due to C_2 symmetry, while the ^{13}C NMR spectrum of **12** contained 21 signals representing all of the carbons in the molecule. The ^{13}C NMR revealed a conjugated ester and a cross-conjugated ketone δ (167.5 and 172.1). Two signals at δ146.7(s) and 129.4(d), suggesting a trisubstituted double bond, were also present. In the ^{1}H-^{1}H COSY spectrum, the partial structures CH(4)-CH(5)-CH(6)-CH(7), and C-H(4′)-CH(5′)-CH(6′)-CH(7′) were revealed. HMBC spectrum indicated the correlation C-3 to H-4, C-3a to H-5, C-7 and C-8 to H-1, C-10 to H-I′, C-11 to H-9 and H-12, C-3′ to H-4′, C-3a′ to H-5, and C-7′ to H-1′ for **12**. This result suggested a seven-member ring for caulersin. Two signals at δ 13.14 and 12.40 indicated that the two NH protons formed hydrogen bonds with two different kinds of carbonyl groups, which supported the structure depicted in **12** [10].

Halimedin (**13**), a 1,3,5-sym-triazine derivative, was isolated from the alga *Helimeda xishaensis,* which is a new species identified by Tseng & Deng. On the basis of spectroscopic methods and X-ray diffraction analysis, the structure of halimedin is determined to be **13**. Halimedin is the first cyano-s-trizine derivative isolated from a natural source [4].



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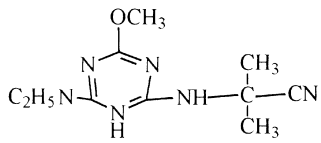

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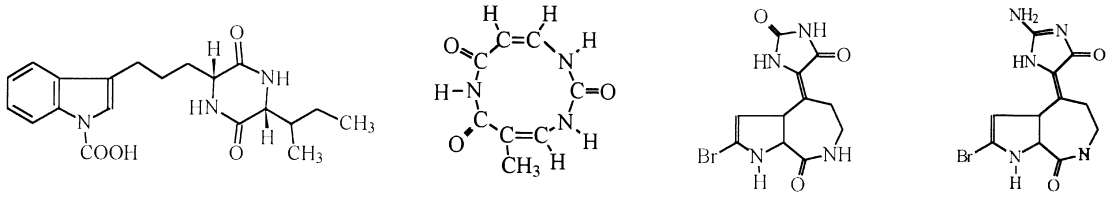
Scheme 4

Pallidin (**14**), an unusual N-carboxyl-indole alkaloid, isolated from marine sponge *Rhaphisia pallida* The ^{13}C NMR DEPT and FABMS (MH^{+}, *m/z* 372) of **14** supported the molecular formula $C_{20}H_{25}N_3O_4$. The pattern of ^{1}H NMR signals in the aromatic region indicated an indole moiety and the four signals at δ 7.34, 7.41, 7.63 and 8.86 ppm were attributed to the four neighboring aromatic protons. The ^{13}C NMR signal at 166.2 ppm and IR absorption at 1700 cm^{-1} established the presence of a COOH group. A typical N-carboxyindole UV absorption strongly supported this determination. The ^{13}C NMR signal at 131.2(d) ppm was assigned to C-2 of indole. The remaining part of the structure was a diketopiperazine system by elucidation of its IR, and ^{1}H NMR data. The interpretation of the ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra established the presence of the following three subunits: –CH=CHCH=CH–, $–CH_2CH_2CH_2CH(N)–$ and $–CH(N)CH(CH_3)CH_2(CH_3)$. The connectivities of these subunits were performed by analyzing the cross-peaks observed in the HMBC spectrum and by comparing the NMR data with those of the known compound ausamide. NOESY experiments were employed to assign the relative configuration for **14** [12] (Scheme 5).

Rhapallin A (**15**) was isolated from the sponge *Rhaphisia pallida* Ridley [13]. Its structure was determined by spectroscopic methods. Rhapallin A have an unusual 10-member heterocyclic ring combining a urea unit with a diacylimide moiety. This is the first report of such a skeleton.

More than 20 new polyhydroxylated sterols have been found from the soft corals and marine sponges. Compound (**18**) exhibited strong anti-inflammatory activity. Sterols (**19**,**20**) showed potent cytotoxicity toward P388 and KB cells.

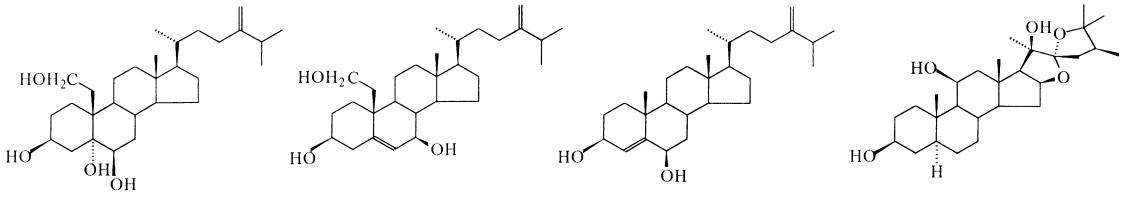
Two rare 19-hydroxyl sterol, nephalsterol A (**18**) and B (**19**) were isolated from the soft corals,



14 15 16 17

Scheme 5

Nephthea albida and *Nephthea tiexieral verseveldt* [14]. The molecular formula of **18** was established as $C_{28}H_{48}O_4$ on the basis of MS and ^{13}C NMR DEPT spectral analysis. In comnparison the ^1H and ^{13}C NMR data of nephalsterol A (**18**) with those of numersterol A (**18A**) clearly revealed that **18** and **18A** have the same sterol side chain structure. ^{13}C NMR showed that **18** contained one primary hydroxyl group, two secondary hydroxyl group and one tertiary hydroxyl group. Since the signal δ_c 19.0 (q, 19-CH_3) was absented in the ^{13}C NMR of **18**, it is reasonable to propose that C-10 position of **18** connected a CH_2OH but not CH_3 group. The remaining three hydroxyl groups were determined by ^1H-^1H, ^1H-^{13}C COSY and LR-CH-COSY NMR to be located at C-3, C-5 and C-6. Their stereochemistry was determined by NOESY experiment.



18 19 20 21

Scheme 6

The new sterol (**20**) was isolated from the soft coral *Alcyonium patagonicum*. It exhibits potent cytotoxic to murine leukemia cells (P388) ($IC_{50} = 1$ μg/mL). Sterol (**20**) displayed M^+ *m/z* 414.3484 by HREIMS, corresponding, to $C_{28}H_{46}O_2$. ^1H and ^{13}C NMR data showed that **20** contained two secondary hydroxyl groups, one terminal and one trisubstituted double bond. The presence of the conventional 24-methylene sterols side-chain was indicated by comparison of ^1H and ^{13}C NMR data with those of the 24-methlylene sterols. On the basis of ^1H-^1H, ^1H-^{13}C COSY and HMBC experiments, The remaining two hydroxyl groups was deduced to be placed at C-3 and C-6. The trisubstituted double bond located in C-4 and C-5. The hydroxyl groups at C-3 and C-6 were assigned as β-configuration based on the coupling constants and NOESY. Thus, the structure of **20** was assigned as 24-methylene-cholest-4-ene-3β,6β-diol [15].

ACKNOWLEDGEMENT

This research were supported by grants from the National Natural Science Foundation, the Natural Science Foundation of Guangdong Province and the Project of Oceanographical Multiplicity Science Expedition of Nansha Islands.

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