海绵甾类化合物研究

V. 海绵 *Pachychalina* sp. 中的甾醇化合物

曾　志*　曾陇梅　苏镜娱

摘　要　从南海海绵 *Pachychalina* sp. 中鉴定出具有 5 种不同甾核的 15 个甾醇，5 种甾核包括 7-烯-甾核，8-烯-甾核，A-降-甾核，5-烯-甾核和 4-甲基胆甾烷醇甾核．2 个在生物合成中具有重要意义的甾醇、24-甲基-25(26)-去氢-胆甾醇和 24-乙烯基胆甾醇，存在于该海锦中．从该海绵中还同时鉴定出 stigmasta-5，24(28)-dien-3β-ol 的 *E* 型和 *Z* 型异构体．用 GC 保留时间和质谱能清晰地区分上述 *E* 型和 *Z* 型异构体．从南海海绵中鉴定出 7-烯-甾核、8-烯-甾核和 4-甲基胆甾烷醇甾核的甾醇，这是首次报导．

关键词　海绵，甾醇，色—质联用

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Studies on the Steroids of Marine Sponge

V. Steroids from the Marine Sponge *Pachychalina* sp.

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Abstract The marine sponge *Pachychalina* sp. collected from the South China Sea has been shown to contain fifteen sterols with five different nuclei including 7 - en - sterols, 8 - en - sterols, A - nor - sterols, 5 - en - sterols and sterols with 4 - methyl cholestanol nuclei. Two of the biosynthetically significant marine sterols, 24 - methyl - 25 (26) - dehydro - cholesterol and 24 - vinylcholesterol, were found in this marine sponge. The *E* - and *Z* - isomer of stigmasta - 5, 24 (28) - dien - 3 β - ol have been identified from the same sponge. The *E* - and *Z* - isomer have clearly distinguished with their GC retention time and mass spectrometry analysis. The occurrence of 7 - en - sterols, 8 - en - sterols and the sterols with 4 - methyl cholestanol nucleus was first uncovered from the South China Sea sponge.

Keywords marine sponge, sterol, GC - MS

1 Introduction

Sterols occur widely in animals as well as in plants, and the role of such compounds in living organisms is being increasingly studied. The extensive researches of Bergmann on the sterols in sponges were carried out before the introduction of efficient chromatographic methods, and he was aware that much of the data accumulated probably referred to sterol mixture and not to individual compounds. Thus most of the earlier work now requires revision with the aid of modern techniques. Although about 5 000 species of sponge can be found in the South China Sea, virtually nothing is known of their sterol contents. This, no doubt, stems from the fact that the great majority are rather small and either rare, or at best, locally abundant, and the difficulties of collection are considerable. We report here on fifteen sterols with five different nuclei detected from the marine sponge *Pachychalina* sp. collected from the South China Sea by GC - MS analysis.

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2 Results and Discussion

The presence of A－nor－sterols in marine sponge from the South China Sea were noted[1]. We now present the separation and identification of three A－nor－sterols, two 7－en－sterols, two 8－en－sterols, six 5－en－sterols and two sterols with 4－methyl cholestanol nucleus from the marine sponge *Pachychalina* sp.

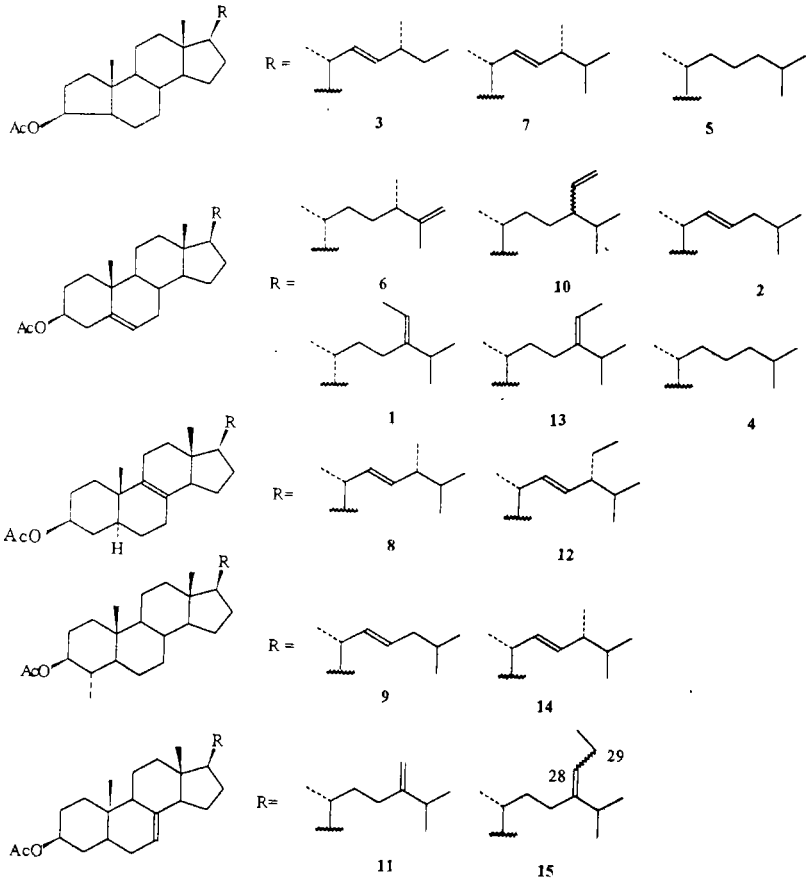

Fig. 1　The structural formulae of the acetates of sterols

2.1　7－En－sterols (as the acetates 11, 15)

It was typical of 7－en－steryl acetate that a very strong peak at m/z 313 was present, sometimes it became a base peak[2]. The presence of a molecular ion peak also established a 7－en－steryl acetate since 5－en－steryl acetate readily lose acetate and did not give molecular ion peaks. The peak at m/z 229 also supported the 7－en－steryl acetate structure. 11 proved to be a C_{28} diunsaturated steryl acetate, which was comfirmed by a molecular ion peak at m/z 440 in the mass spectrum. Further major peaks at m/z 255 and 213 showed that the side chain was C_9 and mono－unsaturated. The peak at $m/$

z 356 corresponding to cleavage $C_{22}-C_{23}$ bond comfirmed the presence of the C_{22} double bond. A very strong peak at m/z 313 (89%) and a peak at m/z 229 indicated the 7 - en - steryl acetate structure. 15 was a C_{30} steryl acetate. The mass spectrum showed a (M^+ - AcOH) peak at m/z 408. A prominent ion peak occurring at m/z 313 and a peak at m/z 229 were characteristic of a 7 - en - steryl acetate. The peak at m/z 356 corresponded to cleavage $C_{22}-C_{23}$ bond from the molecular ion.

2.2 **8 - En - sterols (as the acetates 8, 12)**

The mass spectrum analysis of 8 and 12 showed the major peaks at m/z 380 and 394 respectively corresponding to (M^+ - AcOH). They indicated a C_{28} diunsaturated steryl acetate and a C_{29} diunsaturated steryl acetate. The peak of mass spectra of both 8 and 12 at m/z 282 suggested 22 - en - steryl acetates. All other peaks at $m/z>200$ were of low intensity indicating that they both are 8 - en - steryl acetates because it was typical of 8 - en - steryl acetate[3].

2.3 **A - Nor - sterols (as the acetates 3, 7, 5)**

The mass spectra of 3 and 7 displayed a group of characteristic peaks at m/z 344, 329, 315, 285, 284 and 269. It was typical of 22 - en - A - nor - steryl acetates[4]. The mass spectrum of 3 showed a parent ion peak at m/z 428 suggesting a C_{27} mono - unsaturated steryl acetate. The significant peaks at m/z 257 and 215 and appearance of a weak intensity peak at m/z 413 and the absence of a peak at m/z 385 supported the structure of 3. The mass spectrum of 7 gave a molecular ion peak at m/z 442 suggesting the presence of a C_{28} mono - unsaturated steryl acetate. The presence of saturated sterol nucleus with a monounsaturated side chain was supported by a major peak at m/z 257 and additional peak at m/z 215. The mass spectrum of 5 showed the diagnostic peaks at m/z 276, 275, 230 and 215. They were typical of acetate of A - nor - sterol with a saturated side chain[4]. 5 proved to be the C_{27} steryl acetate, which was confirmed by a molecular ion peak at m/z 430. The other intense peaks at m/z 415, 370, 355, 257 and 215 gave further support for the structure of 5.

2.4 **5 - En - sterols (as the acetates, 1, 13, 6, 10, 2, 4)**

Both 1 and 13 have the same mass spectrum except having consistent differences in the relative intensities of certain ions. They were the E - and Z - isomer of stigmasta - 5, 24 (28) - dien - 3 β - yl acetates. 1 must be the E - isomer and 13 must be the Z - isomer because the E - isomer was eluted before the corresponding Z - isomer in all cases[5]. Table 1 lists the principal ions with relative abundances for the mass spectra for the two compounds analyzed and recorded in literature[5,6].

The mass spectrum analysis of 6 displayed a characteristic peak at m/z 380 corresponding to that for loss of acetic acid from the molecular ion. It indicated a C_{28} di - unsaturated steryl acetate. The major peaks at m/z 255 and 213 showed an acetate of

Tab. 1　Comparison of principal ions in the mass spectra of stigmaste - 5, 24 (28) - dien - 3 β - yl acetates

m/z	$E^{[5]}$	$Z^{[5]}$	1	13	*m/z*	$E^{[5]}$	$Z^{[5]}$	1	13
394 (M^+-60)	73	24	10	11	253	14	10	15	11
379 ($M^+-60-15$)	9	3	2	1	228	12	9	12	11
296 ($M^+-60-98$)	100	100	100	96	213	17	11	18	17
281	20	17	25	20	55	97	60	97	100

sterol with 5 - en - sterol nucleus. The diagnostic peaks at *m/z* 310, 296 and 282 allowed placement of a double bond at the $C_{25(26)}$ position[6,7]. **10** was a C_{29} steryl acetate. The mass spectrum showed a (M^+ - AcOH) peak at *m/z* 394. The prominent peak at *m/z* 255 and 213 displayed an acetate of sterol with 5 - en - sterol nucleus. The diagnostic peaks at *m/z* 310, 296 and 282 were typical of 5, 25 (26) - dien - steryl acetate and 5, 28 (29) - dien - steryl acetate. The other two peaks at *m/z* 379 and 351 corresponding to that for loss of a methyl group and a terminal isopropyl group, respectively, indicated the double bond must be located at $C_{28(29)}$ position[6,7]. The mass spectrum of **2** gave a major peak at *m/z* 366 corresponding to (M^+ - AcOH). The characteristic peak at *m/z* 282, formed by fission of C_{20} - C_{22} bond with one hydrogen transfer, and 255, 213 suggested an acetate of 5, 22 - dien - steryl acetate and a strong peak at *m/z* 111 was typical of a C_8H_{15} side chain steryl acetate containing a C_{22} double bond[8]. The mass spectrum of **4** displayed a strong peak at *m/z* 368 corresponding to (M^+ - AcOH). It indicated a C_{27} mono - unsaturated steryl acetate. The characteristic peaks at *m/z* 255 and 213 indicated an acetate of sterol with 5 - en - sterol nucleus.

2.5　4 - Methyl cholestanols (as the acetates **9**, **14**)

The mass spectrum of **9** had a parent ion peak at *m/z* 442, suggesting the presence of a C_{28} mono - unsaturated steryl acetate. A group of peaks at *m/z* 358, 343, 329, 315, 298 and 283 displayed the presence of acetate of sterol with 4 - methylcholestanol nucleus. The strong peak at *m/z* 358 corresponding to the fission of C_{20} - C_{22} bond with one hydrogen transfer showed a C_{22} double bond structure[8]. The mass spectrum of **14** gave a molecular ion peak at *m/z* 456, which showed a C_{29} monounsaterated steryl acetate. The peaks at *m/z* 315, 298 and 283 also suggested acetate of sterol with 4 - methyl - cholestanol nucleus and C_{22} double bond structure[8].

3　Experimental

3.1　Extraction, isolation and acetylation of the sterol mixture

The marine sponge *Pachychalina* sp. was collected from the South China Sea, in April of 1991. After storage in ethanol, the sponge was homogenized in ethanol and the mixture was filtered. The filtrate was evaporated and the residue dissolved in ethyl ac-

etate. The material on the filter was extracted at room temperature with ethyl acetate and filtered. The extract was combined, washed with water and evaporated to give an extract which was subjected to silica gel column and eluted with petroleum ether containing increasing amounts of ethyl acetate (0%～100%). The sterol fraction was recrystallized from acetone and was then acetylated with Ac_2O in pyridine at 90℃ maintaining 1 h to give the steryl acetate mixture.

3.2 GC－MS condition

GC－MS Analysis was performed on a FINNIGAN GC－MS－4515 instrument with an electron ionization energy of 70 eV and ion current 250 μA and using a DB_5 column (0.25mm×50m). Helium was used as the carrier at a flow rate of 1 mL/min. The temperature was 80℃ maintaining 5 min and 80～150℃, 10℃/min and 150～290℃, 4℃/min. The steryl acetate mixture was dissolved in 10 mL CH_2Cl_2 and the sample of 1 μL solution was introduced on the column.

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References

1 Zeng Longmei, Fu Xiong, Su Jingyu. Studies on the chemical constituents of the South China Sea sponge (Ⅱ). Chem J Chinese Univ, 1991, 12 (7): 924

2 Sica D, Simone F D, Zollo F. Sterols from the sponge Chondrilla nucula. Gazz Chimi Itali, 1978, 108: 575

3 Kokke W C M C, Shoolery J N, Fenical W, et al. Biosynthetic studies of marine lipids. 4. Mechanism of side chain alkylation in (*E*) －24－propylidenecholesterol by a chrysophyte alga. J Org Chem, 1984, 49: 3742

4 Bohlin L, Sjostrand U, Djerassi C, et al. Minor and trace sterols in marine invertebrates. Part 20. 3ξ－Hydroxy－methyl－A－nor－patinosterol and 3ξ－hydroxymethyl－A－nor－dinosterol, two new sterols with modified nucleus and side chain from the sponge Teichaxinella morchella. J Chem Soc Perkin Trans I, 1981, (4): 1023

5 Brooks C J W, Knights B A, Sucrow W, et al. The characterisation of 24－ethylidene sterols Steroids, 1972, 20: 487

6 Catalan C A N, Kokke W C M C, Duque C, et al. Synthesis of (24R)－and (24S)－5, 28－stigmastadiene－3β－ol and determination of the sterochemistry of their 24－hydroxy analogs, the saringosterol. J Org Chem, 1983, 48: 5207

7 Giner J－L, Djerassi C. Biosynthetic studies of marine lipids 31. Evidence for a protonated cyclopropyl intermediate in the biosynthesis of 24－propylidenecholesterol. J Am Chem Soc, 1991, 113: 1386

8 Benveniste P, Hirth L, Ourisson G, La biosynthese des sterols dans les tissus de tabac cultives in vitro. I. Isolement de sterols et de triterpenes. Phytochem, 1966, 5: 31