Phytochemistry, Vol 22, No 5, pp 1239–1240, 1983
Printed in Great Britain

0031-9422/83/051239-02$03 00/0

STEROLS OF THE RED ALGA *RISSOELLA VERRUCULOSA*

SALAM ABDOU KABORE, GEORGES COMBAUT*, JEAN-PIERRE VIDAL, LOUIS CODOMIER*, JEAN PASSET, JEAN-PIERRE GIRARD and JEAN-CLAUDE ROSSI

Groupe de Recherches en Biologie et Chimie des Végétaux Marins, Faculté de Pharmacie de Montpellier, *Université de Perpignan, France

(*Received* 22 *June* 1982)

Key Word Index—*Rissoella verruculosa*, Rhodophyceae; sterols, desmosterol; 24,25-epoxycholesterol.

Abstract—The Mediterranean red alga *Rissoella verruculosa* contains desmosterol in the esterified form whereas this sterol is not present in the free state. Side chain hydroxylated sterols and 24,25-epoxycholesterol were identified for the first time from a natural source by GC/MS analysis of the free sterols

INTRODUCTION

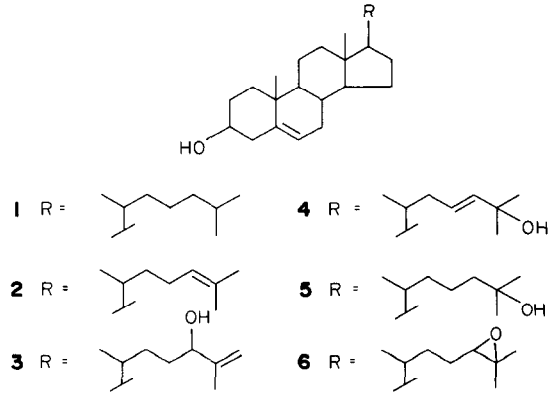
In the course of our investigations on marine algae, we examined the red alga *Rissoella verruculosa* and we now describe the results of the sterol analysis.

Because desmosterol is present in red algae this study was conducted carefully in order to observe any variations between the compositions of the free and esterified sterols and also to determine the presence of minor side chain hydroxylated sterols as reported for *Rhodymenia palmata* and *Asparagopsis armata* [1, 2].

RESULTS

A sample of *Rissoella verruculosa* was collected in June 1981 at Collioure near Perpignan and compared to a sample of *Rissoella verruculosa* taken from Port-Cros, near Marseille [3] The usual work-up including basic hydrolysis and TLC on Si gel yielded a sterol fraction which was analysed by GC and GC/MS of the TMSi derivatives.

Cholesterol (**1**), desmosterol (**2**) and 24-methylcholesta-5,22-dien-3β-ol (brassicasterol or crinosterol) were identified, the two C-27 sterols representing 98% of the total amount of sterols (**1** 40%, **2** 58%). These results are in good agreement with those previously reported [3] (**1** 52%; **2** 48%).



Neither cholesta-5,25-diene-3β,24-diol (**3**) nor liagosterol (**4**) were identified, contrary to the analysis of other red algae in which desmosterol was shown to be present [1, 2, 4]. Analysis of the free sterols isolated by TLC of the crude lipid extract demonstrated that desmosterol was absent. Cholesterol (M.U.31.80) was the major sterol (57%) and, apart from sitosterol (M.U.33.70; 2%), the other sterols (23%, 2% and 16%, respectively) were side chain oxygenated sterols. They were identified by GC/MS of the TMSi derivatives (M.U.32.44, 34.00, 34.33) as 3β-hydroxy-Δ^5-steroids on the basis of a strong *m/z* 129 fragment [5–7].

The sterol TMSi derivative of M.U.32.44 displayed a molecular ion having a mass of 546, with a base peak at *m/z* 73 and a strong *m/z* 129 peak (60%). The fragments at *m/z* 343 (70% $[M - TMSOH - \text{side chain} - 2H]^+$ (70%) and *m/z* 145 (15%) and cleavage of the C-23, C-24 bond confirmed that the sterol was 25-hydroxycholesterol (**5**). The molecular ion at *m/z* 544 of the TMSi derivative of M.U.34.33 and the base peak at *m/z* 143 identified cholesta-5,25-diene-3β, 24ξ-diol (**3**) in *Rissoella verruculosa*. These two sterols were isolated previously from the red alga *Asparagopsis armata* [2] and the reported mass spectral and GC data (M.U. 32.38 and 34.30, respectively) agreed with the present results. However, they represented 23% and 16%, respectively, of the sterol fraction from *Rissoella verruculosa* but they were minor sterols (4% each) in *Asparagopsis armata*.

The TMSi derivative of M.U.34.00 displayed a base peak at *m/z* 73 and a strong *m/z* 129 fragment (56%). The peak at *m/z* 470 (5%) was considered to be the molecular ion while the ion at *m/z* 380 (12%) was the $[M - TMSOH]^+$ fragment. This excludes a hydroxyl group from the side chain such as is found in 25-hydroxy-24-methylcholesterol which has previously been identified from *Asparagopsis armata*. The GC characteristics of this compound (M.U.34.00) were also inconsistent with it being a C_{28} sterol, such as brassicasterol (M.U.32.10 M^+ at *m/z* 470) or 24-methylenecholesterol (M.U.32.60 M^+ at *m/z* 470).

When desmosterol was exposed to air autoxidation for 6 months, all of the material was decomposed and the GC/MS analysis of the TMSi derivatives revealed the

sterols **3** and **5** in the mixture together with the compound of M U 34 00 which has been identified [2] as 24,25-epoxycholesterol (**6**). It is, thus, concluded that the compound of M U 34 00 in the sterol mixture of *Rissoella verruculosa* is **6**

DISCUSSION

Desmosterol has previously been identified in some red algae after using extraction procedures which include basic hydrolysis We have now shown that, in the alga *Rissoella verruculosa*, desmosterol was absent in the free state but was present only in the esterified form Similar results were obtained with a desmosterol-deficient Mediterranean red alga *Asparagopsis armata*, in which desmosterol (10% of the sterol fraction) was identified only when basic hydrolysis was used during the isolation procedure [Combaut, G, unpublished result]

The present results pose several questions regarding the sterols found in marine red algae Can these results be repeated with other alga containing desmosterol? On the other hand, could there be seasonal variations in the concentration of free sterols, as previously reported for the total sterols of *Rhodymenia palmata* [8]? Could the hydroxysterols, **3** and **5**, and the epoxide, **6**, be artifacts due to autoxidation of desmosterol or significant natural products? All of these questions will have to be answered by further investigations of seasonal variations, especially to confirm the absence of desmosterol in the free state during the entire development period of alga

EXPERIMENTAL

Extraction Dried *Rissoella verruculosa* (214 g) was extracted with CH_2Cl_2 (3 × 1 l) yielding a lipid extract (992 mg, 0 48%)

The lipid extract (661 mg) was saponified in KOH–EtOH with reflux for 3 hr and extracted with Et_2O The sterols were isolated by TLC on Si gel (pentane–EtOAc, 7 3) and purified by digitonin pptn (0 028%) Free sterols were isolated without saponification, 331 mg of lipid yielding 18 5 mg of sterols The TMSi derivatives were prepared by treating a soln of sterols with bis(TMS)-acetamide and TMCS (8 2, 65 , 15 hr) [9] GC/MS analysis was performed at 70 eV using a 10 m capillary glass OV-1 column, temp program 180–280° at 2°/min M U calculated by adding a mixture of C_{24}-C_{34} *n*-alkanes to the injected samples

Autoxidation Crystalline desmosterol was separated by HPLC (30 cm × 4 mm i d micro-Bondapak C_{18} column eluted with MeOH) and was then exposed to air for 6 months GC/MS analysis of this sample showed that all of the material had been decomposed Identification of each sterol was made by comparing the MS data of authentic samples [2]

REFERENCES

1 Morisaki, N, Kidooka, S and Ikekawa, N (1976) *Chem Pharm Bull* **24**, 3214
2 Francisco, C, Combaut, G, Teste, J Turchine, C and Djerassi, C (1979) *Steroids* **34**, 163
3 Artaud, J, Iatrides M C, Tisse C, Zahra, J P and Estienne, J (1980) *Analysis* **8**, 277
4 Fattorusso, E, Magno, S, Santacroce, C, Sica, D, Impellizzeri, G, Mangiafico, S, Piattelli M and Sciuto, S (1976) *Biochem Syst Ecol* **4** 135
5 Diekman, J and Djerassi, C (1967) *J Org Chem* **32**, 1005
6 Brooks, C J W Horning, E C and Young, J S (1968) *Lipids* **3**, 391
7 Idler, D R, Khalil M W, Gilbert, J D and Brooks, C J W (1976) *Steroids* **27**, 155
8 Idler, D R and Atkinson, B (1976) *Comp Biochem Physiol B* **53**, 517
9 Francisco, C, Combaut, G, Teste, J and Maume, B F (1977) *Biochim Biophys Acta* **487**, 115