11 Cox, T.M., Mazurier, J., Spik, G., Montreuil, J., and Peters, T.J., Iron binding proteins and influx of iron across the duodenal brush border. Evidence for specific lactotransferrin receptor in the human intestine. Biochim. biophys. Acta *588* (1979) 120–128.
12 Doolittle, R.F., Woodbury, N.W., and Jue, R.A., Ribosomal protein S1 is the product of a serie of contiguous duplications. Biosc. Rep. *2* (1982) 405–412.
13 Elleman, T.C., and Williams, J., The amino acid sequences of cysteic acid-containing peptides from performic acid-oxidized ovotransferrin. Biochem. J. *116* (1970) 515–535.
14 Gorinsky, B., Horsburgh, C., Lindley, P.F., Moss, D.S., Parkar, M., and Watson, J.L., Evidence for the bilobal nature of differic rabbit plasma transferrin. Nature *281* (1978) 157–158.
15 Jandl, J.H., Imman, J.K., Simmons, R.L., and Allen, D.W., Transfer of iron from serum iron-binding protein to human reticulocytes. J. clin. Invest. *38* (1959) 161–185.
16 Jeltsch, J.M., and Chambon, P., The complete nucleotide sequence of the chicken ovotransferrin mRNA. Eur. J. Biochem. *122* (1982) 291–295.
17 Krysteva, M., Mazurier, J., Spik, G., and Montreuil, J., Comparative study on histidine modification by diethylpyrocarbonate in human serotransferrin and lactotransferrin. FEBS Lett. *56* (1975) 337–340.
18 McGillivray, R.T.A., Mendez, E., and Brew, K., Structure and evolution of serum transferrin, in: Proteins of iron metabolism, pp.133–141. Eds E.B. Brown, P. Aisen, J. Fielding, and R.R. Crichton. Grune and Stratton, New York 1977.
19 McGillivray, R.T.A., Mendez, E., Sinha, S.K., Sutton, M.R., Lineback-Zins, J., and Brew, K., The complete amino acid sequence of human serum transferrin. Proc. natl Acad. Sci. USA *79* (1982) 2504–2508.
20 Mazurier, J., Léger, D., Tordera, V., Montreuil, J., and Spik, G., Comparative study of the iron-binding properties of transferrins. Differences in the involvement of histidine residues as revealed by carbethoxylation. Eur. J. Biochem. *119* (1981) 537–543.
21 Metz-Boutigue, M.H., Jollès, J., Mazurier, J., Spik, G., Montreuil, J., and Jollès, P., Structural studies concerning human lactotransferrin: its relatedness with human serum transferrin and evidence for internal homology. Biochimie *60* (1978) 557–561.
22 Metz-Boutigue, M.H., Jollès, J., Mazurier, J., Spik, G., Montreuil, J., and Jollès, P., Structural relatedness between human lactotransferrin and human ceruloplasmin. FEBS Lett. *132* (1981) 239–242.
23 Metz-Boutigue, M.H., Jollès, J., Mazurier, J., Spik, G., Montreuil, J., and Jollès, P., An 88 amino acid long C-terminal sequence of human lactotransferrin. FEBS Lett. *142* (1982) 107–110.
24 Metz-Boutigue, M.H., Mazurier, J., Jollès, J., Spik, G., Montreuil, J., and Jollès, P., The present state of the human lactotransferrin sequence. Study and alignment of the cyanogen bromide fragments and characterization of N- and C-terminal domains. Biochim. biophys. Acta *670* (1981) 243–254.
25 Montreuil, J., and Spik, G., Comparative studies of carbohydrate and protein moieties of human serotransferrin and lactotransferrin, in: Proteins of iron storage and transport in biochemistry and medicine, pp.27–38. Ed. R.R. Crichton. Elsevier Biomedical, Amsterdam-New York 1975.
26 Neuberger, A., and Marshall, R.D., Aspects of the structure of glycoproteins, in: Carbohydrates and their roles, pp.115–132. Eds H.W. Schultze, R.F. Cain, and R.W. Wrolstad. Avi, Westport, Conn., USA, 1969.
27 Rogers, T.B., Børresen, T., and Feeney, R.E., Chemical modification of the arginines in transferrins. Biochemistry *17* (1978) 1105–1109.
28 Ronin, C., Bouchilloux, S., Granier, C., and Van Rietschoten, J., Enzymatic N-glycosylation of synthetic Asn-X-Thr containing peptides. FEBS Lett. *96* (1978) 179–182.
29 Schlabach, M.R., and Bates, G.W., The synergistic binding of anions and $Fe^{3+}$ by transferrin. Implications for the interlocking sites hypothesis. J. biol. Chem. *250* (1975) 2182–2188.
30 Tan, A.T., and Woodworth, R.C., Differences in absorption and emission properties of conalbumin and metal saturated conalbumin. J. Polymer Sci. Part C, (1970) 599–606.
31 Warner, R.C., and Weber, I., The metal-combining properties of conalbumin. J. Am. chem. Soc. *75* (1953) 5094–5101.
32 Williams, J., The formation of iron-binding fragments of hen ovotransferrin by limited proteolysis. Biochem. J. *141* (1974) 745–752.
33 Williams, J., The identification of protected tyrosine residues in iron-ovotransferrin. Biochem. J. *201* (1982) 647–651.
34 Williams, J., Elleman, T.C., Kingston, I.B., Wilkins, A.G., and Kuhn, K.A., The primary structure of hen ovotransferrin. Eur. J. Biochem. *122* (1982) 297–303.

0014-4754/83/020135-07$1.50+0.20/0


# Short Communications

## Further caulerpenyne-like esters from the green alga *Caulerpa prolifera*[1]

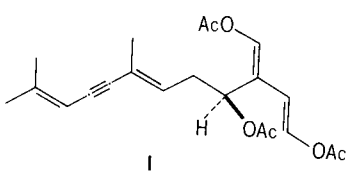
L. De Napoli, S. Magno, L. Mayol and E. Novellino

*Istituto di Chimica Biorganica dell'Università di Napoli, Via L. Rodinò 22, I-80138 Napoli (Italy), June 6, 1982*

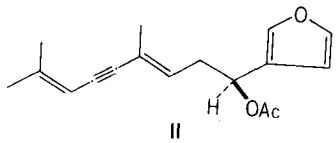
*Summary.* From a further investigation of the extractive of the green marine seaweed *Caulerpa prolifera,* we isolated **III**, which, on the basis of chemical and physico-chemical data, proved to be a dihydroderivative of caulerpenyne with an acetoxy group substituted by fatty acid residues.

Two previous reports from this laboratory[2,3] describe the isolation from *Caulerpa prolifera,* a green marine seaweed widely distributed in Mediterranean waters, of a linear sesquiterpenoid, caulerpenyne (**I**), and of furocaulerpin (**II**), biogenetically related to (**I**).

Structurally related sesquiterpenoids and diterpenoids with antimicrobial and antifeedant activities[4,5] were also found in other species of Chlorophyceae belonging to the same order (Siphonales), and this could have a chemotaxonomic significance. The biological properties of this class of natural compounds prompted us to investigate minor constituents of *C. prolifera* and in this paper we describe the isolation and structure elucidation of further caulerpenyne-like esters from this alga.



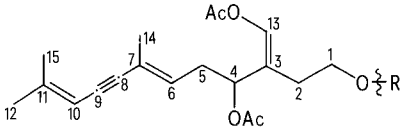
I



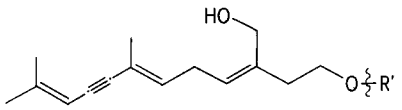
II

*Material and methods.* Samples of *C. prolifera* were collected in the Bay of Salerno, Italy, during autumn 1981. The alga was freeze-dried and repeatedly extracted with $CHCl_3$. The combined chloroform extracts were evaporated to obtain a dark brown gum (22.8 g, 4.2% dry wt).

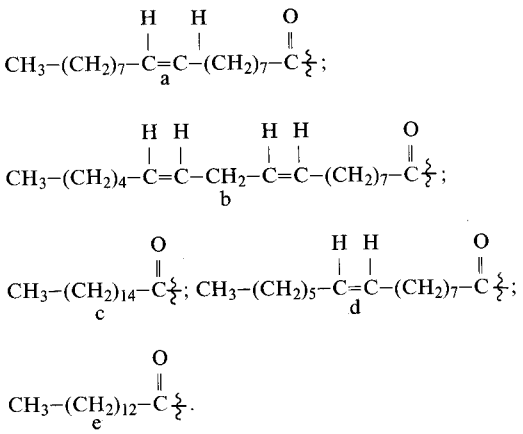
The chloroform extract was chromatographed on a silica gel column using increasing amounts of $Et_2O$ in $C_6H_6$ as eluant. The fractions eluted with $C_6H_6$-$Et_2O$ 96:4 taken to dryness gave an oil (400 mg) which was further chromatographed on a $SiO_2$ column using light petroleum-$Et_2O$ 8:2 as eluant thus obtaining 109 mg of crude **III**, which was purified on a silica gel plate with 4:1 hexane-AcOEt as eluant to give **III** (55 mg, 0.01% yield based on dry wt) as a pale oil.



**III** R = a(23%); b(14%); c(32%); d(9%); e(22%).



**IV** R' = a,b,c,d,e.
**V** R = H



Reduction of **III** was performed by adding $NaBH_4$ (20 mg) to a solution of the mixture of esters **III** (20 mg). After destruction of excess reagent and extraction with $Et_2O$, the crude product was purified by chromatography on a silica gel plate with 4:1 $C_6H_6$-$Et_2O$ as eluant to obtain the mixture of esters **IV** (8 mg).

The mixture of esters **III** was saponified in 10% KOH in 4:1 ethanol-water under reflux for 1 h. The aqueous layer, after acidification and extraction, afforded a mixture of fatty acids which after treatment with $CH_2N_2$ in $Et_2O$ was analyzed by GLC, using a 2.5% OV 17 column, t 200 °C, flow of $N_2$ 30 ml/min. Each component of the mixture was identified on the basis of its retention time and the identifications were confirmed by coGLC with authentic samples.

Reduction of **IV** with $LiAlH_4$ was performed by addition of a solution of the mixture of esters **IV** (10 mg) in anhydrous ether (3 ml) to a stirred suspension of $LiAlH_4$ (50 mg) in anhydrous ether (3 ml) and the reaction mixture was stirred for 2 h at room temperature. A conventional work-up led to the isolation of **V**.

*Results and discussion.* UV-spectra of **III** (*n*-hexane, $\lambda_{max}$ 256, 268 and 283 nm, $E^{1\%}_{1cm}$ 243, 294.7 and 233.6) pointed to the presence of the conjugated system $C_6$–$C_{11}$ which was confirmed by NMR-spectrum performed by a Brücker WH-270 spectrometer using $CDCl_3$ as solvent [3 broad 3H-singlets at $\delta$ 1.89 (11-Me, cis), 1.82 (11-Me, trans) and 1.85 (7-Me), a triplet at $\delta$ 5.70 (1 H, J = 7.0 Hz, 6-H) broadened by long range coupling with 7-Me, and a broad singlet at $\delta$ 5.36 (2.2 H), due to the superimposition of the 10-H signal and of the signals of the olefinic protons of unsaturated fatty acid residues (see below)].

Further information on the structure **III** was obtained from extensive spin decoupling experiments performed on the PMR-spectrum of **III**. In fact the 5-$H_2$ methylene protons appear as 2 symmetrical double double doublets (J = 15.0, 6.5 and 7.0 Hz) at $\delta$ 2.70 and 2.56, coupled with the triplets at $\delta$ 5.70 and 5.89 (1 H, J = 6.5 Hz, 4-H) while 1-$H_2$ methylene group resonates as a multiplet at $\delta$ 4.14, which collapsed into an AB system ($J_{AB}$ = 9.9 Hz) by irradiation at $\delta$ 2.36 (2-$H_2$ frequency).

The presence of the 2 acetoxy groups, one of them attached to a trisubstituted double bond, was deduced from the IR-spectrum, ($\nu^{CCl_4}_{max}$ 1760 and 1740 $cm^{-1}$) and the NMR-spectrum [2 3H-singlets at $\delta$ 2.17 and 2.06 (vinylic and allylic acetate Me groups, respectively) and 7.06 (1 H, bs, 13-H)] which also includes an intense signal at $\delta$ 1.26, indicative of saturated hydrocarbon long chains.

At this juncture it became apparent that **III** is indeed a dihydroderivative of caulerpenyne where an acetoxy group is substituted by fatty acid residues. This was confirmed by the mass spectrum of **III** which includes molecular ions at m/z 598, 596, 572, 570 and 544 and 3 series of peaks deriving from the parent ions by loss of $CH_3COOH$, $CH_3COOH + CH_2CO$ and $CH_3COOH + CH_3CO$ respectively. Further diagnostically important peaks are also present at m/z 316 ($M^+$'s-fatty acids), 256 (316-$CH_3COOH$), 214 (256-$CH_2CO$), 213 (256-$CH_3CO$), 183 (316-$C_{10}H_{13}$), 141 (base peak; 183-$CH_2CO$) and 133 ($C_{10}H_{13}$).

Alkaline hydrolysis of **III** and GLC-analysis of the acidic fraction after $CH_2N_2$ treatment confirmed the spectral data indicating the presence of oleic (23%), linoleic (14%), palmitic (32%), palmitoleic (9%) and myristic (22%) acid methyl esters.

From the above results it could not be established whether the fatty acid residue is on C(1) and consequently, the acetoxyl group on C(4) or vice versa. The 2nd possibility was excluded on the basis of $NaBH_4$ reduction of **III** which afforded the mixture of hydroxyesters **IV** whose structure was deduced from the spectral data [UV (*n*-hexane) $\lambda_{max}$ 256, 268 and 283 nm ($E^{1\%}_{1cm}$ 240, 291 and 220.6); IR ($CCl_4$) $\nu_{max}$ 3210 (OH), 1738 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) 1.81 (3 H, s, 11-Me, trans), 1.84 (3 H, s, 7-Me), 1.89 (3 H, s, 11-Me, cis), 2.90 (2 H, bt, J = 6.8 Hz, 5-$H_2$), 4.07 (2 H, s 13-$H_2$), 4.15 (2 H, m, 1-$H_2$), 5.52 (1 H, t, J = 6.8 Hz, 4-H) and 5.74 (1 H, t, J = 6.8 Hz, 6-H); in the NMR-spectrum an intense signal at $\delta$ 1.26, due to the long chains' methylene protons is also present; MS, m/z 216 ($M^+$'s-fatty acid), 198 (base peak, 216-$H_2O$)].

Decisive proof for the structure of **IV** and, consequently, of **III** was obtained from $LiAlH_4$ reduction of **IV** which afforded **V** identified by comparison with a sample obtained from caulerpenyne by $NaBH_4$ reduction[2]. This result enables us to assign the *E* configuration to C(6) double bond.

The *Z* configuration of the C(3) double bond in **III** was established by application of nuclear Overhauser effect: irradiation at $\delta$ 7.06 (13-H frequency) resulted in a 12% enhancement of the integrated adsorption of the methylene protons at C(2) whereas the 4-H signal was not significantly affected.

1 This work was supported by CNR, Rome, in the framework of 'Progetto Finalizzato Chimica Fine e Secondaria'.
2 Amico, V., Oriente, G., Piattelli, M., Tringali, C., Fattorusso, E., Magno, S., and Mayol, L., Tetrahedron Lett. *1978*, 3593.
3 De Napoli, L., Fattorusso, E., Magno, S., and Mayol, L., Experientia *37* (1981) 1132.
4 Blackman, A.J., and Wells, R.J., Tetrahedron Lett. *1978*, 3063.
5 Sun, H.H., and Fenical, W., Tetrahedron Lett. *1979*, 685.

0014-4754/83/020141-03$1.50+0.20/0


## Increase in micropore volume of N-containing activated carbon treated with methylol melamine urea solution

K. Boki[1], S. Tanada, T. Kita, T. Nakamura, H. Takahashi and R. Hamada

*Faculty of Pharmaceutical Sciences, Tokushima University of Arts and Sciences, Yamashiro-cho, Tokushima 770 (Japan), and Faculty of Pharmaceutical Sciences, Faculty of Agriculture, Kinki University, Kowakae 3-4-1, Higashi-Osaka, Osaka 577 (Japan), February 26, 1982*

*Summary.* The micropore volume of N-containing activated carbon was increased and the average radius of supermicropore was extended by treatment with methylol melamine urea solution.

The theory of volume filling of micropores (TVFM)[2] is applied for describing the physical adsorption of gas in micropores. The adsorption for micropores (radius < 5–6 Å) and supermicropores (5–6 < radius < 15–16 Å according to TVFM is expressed by the two-term equation[3],

$$a = W_{01}/\mu * \exp[-(A/\beta E_{01})^2] + W_{02}/\mu * \exp[-(A/\beta E_{02})^2],$$

where a is the amount adsorbed; $\mu *$ is the molar volume of an adsorbate; $W_{01}$ and $W_{02}$ are the micropore and the supermicropore volumes, respectively; A is the decrease of free energy of adsorption; $E_{01}$ and $E_{02}$ designate the characteristic energies of adsorption in micropores and supermicropores, respectively; and $\beta$ is the similarity coefficient.

In the previous paper[4] it was demonstrated that the N-containing activated carbon (N-CAC) prepared with methylol melamine urea (MMU) solution had the highest adsorption capacity for hydrogen sulfide at pressures up to about 400 Torr among the 20 kinds of N-CACs. N-CAC would be of great value for a large scale utilization because of the effects of its molecular sieving nature[5] and its surface polar nature[5]. The present investigation was undertaken to describe the difference in porous structure between the raw activated carbon and the N-CAC prepared with MMU solution on the basis of the results of application of the two-term equation to the experimental isotherms of hydrogen sulfide on them.

*Materials and methods.* The purity of hydrogen sulfide gas was indicated to be 99.9%. The physical properties of raw activated carbon (No. 1) and N-CAC prepared with MMU

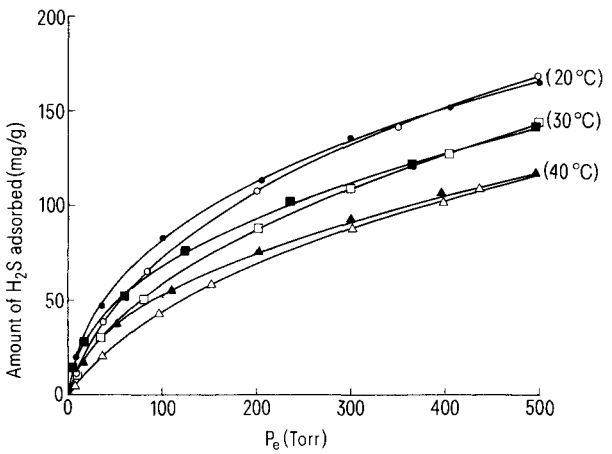



Figure 1. Adsorption isotherms of hydrogen sulfide on activated carbon. Open symbols and closed symbols denote the experimental data of adsorption on activated carbon Nos 1 and 2, respectively. The equilibrium amounts adsorbed at different equilibrium pressures were determined within an error of 0.5%. $P_e$ is the equilibrium pressure of hydrogen sulfide.

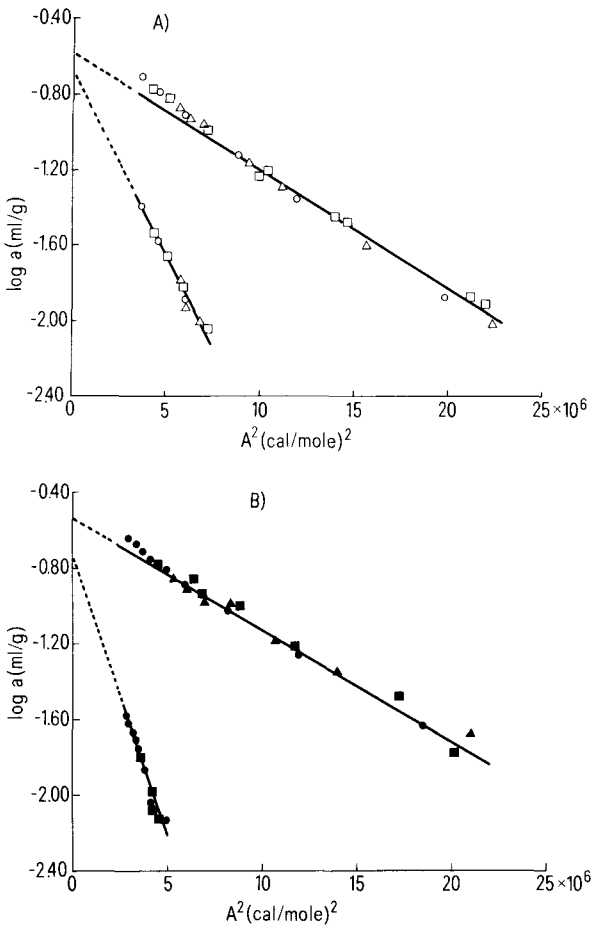



Figure 2. Application of the Dubinin-Radushkevich equation and the two-term equation to the experimental adsorption isotherms of hydrogen sulfide. *A* Activated carbon No. 1; *B* activated carbon No. 2; a, the amount of hydrogen sulfide adsorbed (ml/g); A, the decrease of free energy of adsorption.