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Gonyol: Methionine-Induced Sulfonium Accumulation in a Dinoflagellate *Gonyaulax polyedra*

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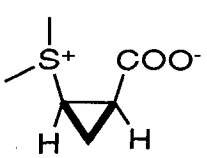
Keywords: Gonyol; Methionine-induced; Gonyaulax polyedra; Dinoflagellate; Biogenesis

Abstract: *Gonyol, 3S-5-dimethylsulfonio-3-hydroxylpentanoate, was isolated from a dinoflagellate Gonyaulax polyedra cultured in a medium containing methionine and the structure was elucidated by spectroscopic method and confirmed by chemical synthesis. Gonyol was shown to be biogenetically derived from methionine and acetic acid.*

Unicellular algae as well as marine and terrestrial plants produce sulfonium compounds such as *S*-methylmethionine (vitamin U) and *S*-adenosylmethionine. Sulfoniums are shown to be a major source of atmospheric sulfur compounds such as dimethyl sulfide and methanthiol from ocean.[1] We reported that a dinoflagellate *Gonyaulax polyedra* contains a large amount of gonyauline (**1**) (about 10 mg/l g wet cells), which shortens a period of bioluminescent circadian rhythm of *Gonyaulax polyedra*.[2] During our course of studies on biogenesis and metabolisms of gonyauline in the cells, we found that a new sulfonium compound named gonyol was accumulated in the cells when methionine was added to the culture medium at a high concentration. We wish to report the isolation, structure elucidation, synthesis, and biogenesis of gonyol (**2**).



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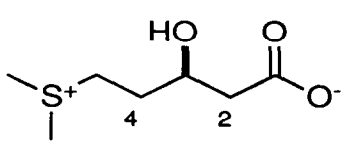
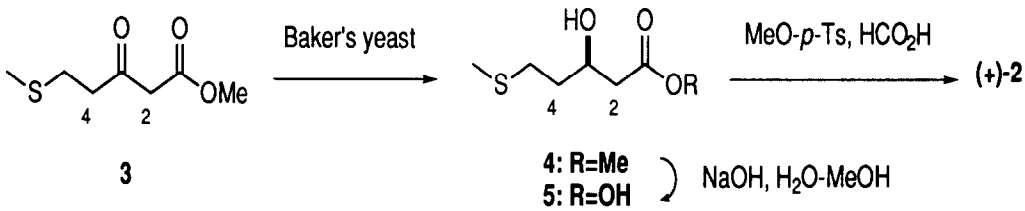


2

Because it was estimated, on the basis of structural similarity between gonyauline and methionine, that gonyauline might be derived directly from methionine by methylation and deamination-cyclopropanation reactions accompanied with an inversion of configuration at C-2 position, we have examined feeding experiments of labelled methionine or its analogues to *G. polyedra*.[3] As a result, methyl groups of methionine or *S*-methylmethionine were efficiently incorporated into a sulfonium group of gonyauline, however, carboxyl carbons of methionine or *S*-methylmethionine at C-1 position were not incorporated into gonyauline even at a high concentration.[4] On the other hand, in the presence of methionine-^{13}C-methyl (10-100 μM), a set of labelled signals for methylsulfonium group (δ_C24.5, 24.7, δ_H2.9) in addition to two for that of gonyauline (δ_C26.8, 26.7, δ_H2.89, 3.01) was observed in the crude water-soluble extracts of cultured *G. polyedra* at about 1:2 ratio (100 μM) whereas almost only the signals for gonyauline were found in the extracts of *G. polyedra* cultured in the normal medium.

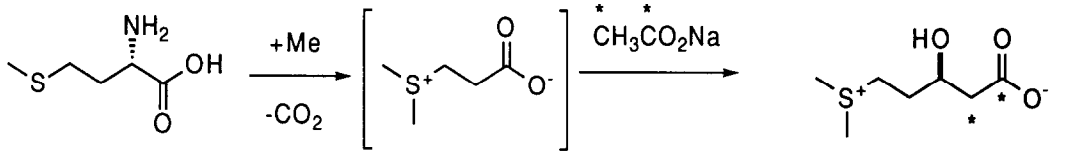
Gonyol was isolated from 70% EtOH extracts of *G. polyedra* (1.26 g wet weight from 2 L x 3 flasks) cultured in a medium containing 100 μM L-methionine by monitoring NMR signals as follows. The extracts were partitioned with water and EtOAc and the water-soluble portion was chromatographed on a cation exchange column (SO_3H form) with hydrochloric acid (0.5 M, 0.8 M, and 1.0 M) as eluate. The 0.8 M HCl fraction was separated on an ODS column (0.1% trifluoroacetic acid) to give **2** as an oil of trifluoroacetic acid salts (4.9 mg, 0.39% isolation yield), $[\alpha]_D^{25}$ +3.7 ° (*c* 0.30, H_2O). HR-EIMS of **2** established a molecular formula of $C_7H_{14}O_3S$ (*m/z* 178.0662, ΔM -0.2 mmu) and the structure was deduced on the basis of ^{1}H and ^{13}C NMR spectra.[5]



The structure of gonyol was confirmed by chemical synthesis started from methyl-5-methylthio-3-oxopentanoate (**3**). Reduction of **3** with Baker's yeast gave 3*S*-hydroxy compound **4** with 20% e.e. as reported by M. Hirama *et al.*[6] The alcohol **4** was hydrolyzed with NaOH in H_2O-MeOH at 0 °C for 1 h to give carboxylic acid **5** (38% yield).[8] The acid **5** was methylated with methyl *p*-toluenesulfonate in formic acid at 35 °C for 3 h , and purified by the method described for natural gonyol to afford pure synthetic **2** (68% yield). The synthetic gonyol showed $[\alpha]_D^{25}$ +0.75 ° (*c* 1.95, H_2O), which corresponds to +3.8 ° of the optically pure compound. These data prove the structure of gonyol as 3*S*-5-dimethylsulfonio-3-hydroxypentanoate.

As same as gonyauline, no carboxyl carbon of methionine was incorporated into gonyol. However the carbons at C-1 and C-2 was labelled (1%) with sodium acetate-$^{13}C_2$ (50 μM) in the presence of methionine (100 μM). The fact suggests that gonyol might be biogenetically derived from methionine and acetate through dimethyl-β-propiothetin (6)[9] or its analogous intermediates. Dimethyl-β-propiothetin was found in several algae including dinoflagellates as a major water soluble component and believed to display important roles such as regulation of cellular osmotic pressure[10] and biomethylation[11] in algae.

Although gonyol was found as a minor component in *G. polyedra* under normal culture conditions, other dinoflagellates such as *Amphidinium* sp. and *Symbiodinium* sp. contained gonyol at a different content from a trace to one of major components. Dimethyl-β-propiothetin might be a common precursor for methionine cascade to sulfonium compounds including gonyauline and gonyol. Further studies on biogenesis of gonyauline and gonyol are in progress at our laboratory.



6 2 * : ^{13}C

Acknowledgments: We are grateful to Prof. J. W. Hastings for kind donation of the *Gonyaulax* strain (E5). We acknowledge the financial support from the Shorai Foundation. This work was partly supported by a Grant-in-Aid from Ministry of Education, Culture and Science.

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3. *G. polyedra* (strain E5) was unialgal but not axenic and the strain used was deposited to the Microbial Culture Collection, the National Institute for Environmental Studies, Tsukuba, Japan. *G. polyedra* was cultured in f/2 medium ($NaNO_3$ 75 mg, NaH_2PO_4-$2H_2O$ 6 mg, vitamin B_{12} 0.5 μg, biotin 0.5 μg, thiamine HCl 100 μg, Na_2EDTA-$2H_2O$ 4.4 mg, $FeCl_3$-$6H_2O$ 3.16 mg, $CoSO_4$-$7H_2O$ 12 μg, $ZnSO_4$-H_2O 21 μg, $MnCl_2$-$4H_2O$ 180 μg, $CuSO_4$-$5H_2O$ 7 μg, and Na_2MoO_4-$2H_2O$ 7 μg in 1

L of 9:1 sea water-deionized water), without silicate but with 0.5% (v/v) soil extract, under a 12:12 light/dark cycle for 3 weeks after inoculation. Chemicals sterilized with a membrane filter (0.25 μm) were added 1week before harvest. Antibiotics (streptomycin 5 mg/L and chloramphenicol 2.5 mg/L) did not affect the results.

4. Several amino acids showed acute toxicity (24 h) at a high concentration. Concentrations for 50% lethal toxicity of methionine and S-methylmethionine were 500 and 50 mM, respectively, in a medium containing 4000 cells/ml under the culture conditions. Radio-labelled methionine was slowly incorporated into *G. polyedra.*
5. **2**: ^{1}H NMR (270 MHz, D_2O) δ1.97 (1H, m, H-4), 2.09 (1H, m, H-4), 2.52 (1H, dd, *J*=8, 16 Hz, H-2), 2.29 (1H, dd, *J*=6, 16 Hz, H-2), 2.94 (6H, s, $S^{+}Me_2$), 3.38 (1H, ddd, *J*=6, 9, 12 Hz, H-5), 3.45 (1H, ddd, *J*=5, 8 , 12 Hz, H-5), 4.18 (1H, m, H-3); ^{13}C NMR (67.5 MHz, D_2O) δ24.4 ($S^{+}Me$), 24.6 ($S^{+}Me$), 29.8 (C-4), 40.0 (C-5), 41.0 (C-2), 66.0 (C-3), 174.9 (C-1).
6. Absolute configuration was reconfirmed by modified Mosher's method[7] to be *S* as reported: Hirama, M., Nakamine, T., and Ito, S., Chem. Lett., **1986**, 1381.
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8. **5**: ^{1}H NMR (270 MHz, $CDCl_3$) δ1.70-1.90 (2H, m, H-4), 2.10 (3H, s, SMe), 2.47-2.60 (2H, m, H-2), 2.63 (2H, m, H-5), 4.21 (1H, m, H-3); HR-EIMS *m/z* 164.0502 ($C_6H_{12}O_3S$, ΔM 0.6 mmu).
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