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β-HYDROXYBUTYRIC ACID POLYMER FROM *HYDROCLATHRUS CLATHRATUS*

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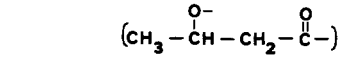
Hydroclathrus clathratus (Bory ex C.A. Agardh) Howe (Punctariaceae) is a common brown alga on the coast of the Red Sea. It is eaten by inhabitants of the Hawaiian Islands (1) and is used as a fertilizer in China and the Phillipines (2).

Alginic acid (1, 3), mannitol (4), and vitamins (1, 5, 6) were reported to be constituents of *H. clathratus*. This communication describes the isolation of a β-hydroxybutyric acid polymer from the Red Sea algae.

RESULTS AND DISCUSSIONS

The $CHCl_3$ extract of the C_6H_6 defatted thalli of the alga afforded, upon standing and precipitation with EtOH, a colorless amorphous residue. The ^{1}H-nmr spectrum of the product showed a doublet at δ 1.28 and two sets of complex multiplets centered at δ 2.55 and δ 5.28. Irradiation at the frequency of the doublet resulted in collapse of the multiplet at δ 5.28 into a triplet (J=7 Hz) but did not affect the multiplet at δ 2.55. On the other hand, irradiation at the frequency of the multiplet at δ 2.55 resulted in collapse of the multiplet at δ 5.28 into a quartet (J=7 Hz), while irradiation of the multiplet at δ 5.28 resulted in collapse of the δ 2.55 multiplet into two doublets (J=13 Hz) and the δ 1.28 doublet into a singlet. The ^{13}C-nmr spectrum showed an ester carbonyl signal (δ 169.2, s), an oxygenated methine carbon (δ 67.7, d), a methylene carbon (δ 41.0, t), and a methyl group (δ 19.8, q). The ester nature of the carbonyl group was confirmed by the presence of a band at 1730 cm^{-1} in the ir spectrum. These data suggested the partial structure **1**.



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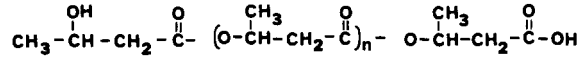
The presence of only four carbons in the ^{13}C-nmr spectrum indicated that the compound was a polymer of β-hydroxybutyric acid. Eims analysis showed fragment ions up to m/z 517, with 86 mass unit intervals down to m/z 87, supporting the polymeric nature of the molecule. The fact that the compound was a long-chain polyester and not a cyclic structure was deduced from the presence of significant ions showing loss of H_2O from the major fragments in the ms. Thus, the compound was assigned structure **2**.

Base hydrolysis of the polymer resulted in the formation of crotonic acid as the only identifiable product by gc/ms of the trimethylsilyl derivative.

Attempts to determine the MW of the polymer using fdms or fabms was unsuccessful. Thus, at this point, the length of the polymer chain is undetermined.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were carried out on a hot-stage Kofler microscope and are uncorrected. ^{1}H-nmr spectra were recorded in $CDCl_3$ using a Varian EM-390 90 MHz instrument. ^{13}C-nmr spectra were obtained on a JEOL NJM-FX-60 Fourier transform instrument. Chemical shifts were measured in δ-values (ppm) with TMS as internal standard. A Finnigan 3200 GC/MS/DS system was used for eims, and a Varian-MAT 731 instru-



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ment was used for fdms analysis at emitter current of 17-20-mA.

ALGAL MATERIAL.—Thalli of *H. clathratus* were collected from coral reefs of Abu-Sadafa, El-Gardaga, Egypt, in April 1981. Herbarium specimens were identified by Profs. G. Furnari and M. Cormaci from the University of Catania. Voucher specimens are kept at the Museum of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt. Fresh algal thalli were cleaned from foreign matter, washed with H_2O, and then air-dried.

ISOLATION.—Powdered thalli (500 g) were packed in a Soxhlet apparatus, defatted with C_6H_6, and then exhaustively extracted with $CHCl_3$. The concentrate obtained after evaporation of the solvent afforded a greenish amorphous deposit on standing, and precipitation was enhanced by the addition of EtOH. Repeated precipitation from $CHCl_3$/EtOH gave a pure, colorless product (30 mg); mp 63-64° $[\alpha]^{25}D$ ±0 (*c* 0.25, $CHCl_3$); ir ν max ($CHCl_3$ cm^{-1} 1730, 1380, 1300, 1180, and 1060; ^1H nmr ($CDCl_3$) δ 1.28 (3H, d, J=7 Hz, 2.55 (2H, m), 5.28 (1H, m); ^{13}C nmr ($CDCl_3$) 169.2 (s), 67.7 (d), 41.0 (t), 19.8 (q); eims *m/z* (%) 517 (0.1), 499 (0.1), 431 (1), 413 (1), 345 (2), 327 (5), 259 (6), 241 (21), 173 (26), 155 (100), 154 (99), 87 (62).

HYDROLYSIS OF THE POLYMER.—Base hydrolysis of the isolated compound (5 mg) was carried out using 2 ml of 2 N alcoholic KOH and heating under reflux for 15 min. The solvent was then evaporated and the residue acidified with 2 N HCl and extracted with Et_2O (2×5 ml). The Et_2O layer was evaporated and the residue reacted with 0.2 ml pyridine and 0.2 ml BSTFA. Gc/ms analysis of the product (30 m×0.25 mm SE-30 capillary column, 100-250° at 10°/min) showed a peak at 1.8 min, identical with that from a standard crotonic acid trimethysilyl derivative [ms *m/z* (%) 158 (0.3), 143 (100%) and 99 (43)].

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