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FICULINIC ACID A AND B, TWO NOVEL CYTOTOXIC STRAIGHT-CHAIN ACIDS FROM THE SPONGE *FICULINA FICUS*

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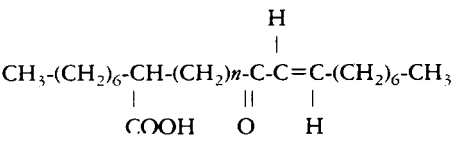
From our continuing program aimed at the isolation of compounds that exhibit antitumor activity and are obtained from marine invertebrates, we report here the investigation of the sponge *Ficulina ficus* Gray (Suberitidae). Structural studies and biological assays have been performed on two minor components, ficulinic acid A and B, which were isolated from a hexane extract of this sponge. This extract has previously been shown to contain large amounts of carotenoids (1) and sterols.[1]

RESULTS AND DISCUSSION

Ficulinic acid A and B were assigned the structures **1** and **2**, respectively, from the spectral evidence shown below; these structures have not previously been reported.



1 n=7
2 n=9

The empirical formula of **1** was determined to be $C_{26}H_{48}O_3$ by hrms, and its ir ($\nu_{C=O}$ = 1640, 1700 cm^{-1}) and uv (λ max = 230 nm) spectra were indicative of a saturated carboxylic acid and of an α,β-unsaturated ketone. In the ^{1}H-nmr spectrum, the chemical shifts and the coupling constants (6.89 and 6.04 ppm, J = 16 Hz) of the ethylenic protons favor a *trans*-double bond α to the ketone carbonyl group rather than to the carboxylic one.

The positions of the functional groups were established from mass spectra and high resolution determinations of the characteristic fragments (Scheme 1). The fragmentations due to McLafferty type rearrangements were particularly helpful. Final confirmation of the position of the double bond was obtained by oxidation with permanganate-periodate (2) and identification of octanoic acid in the product mixture.

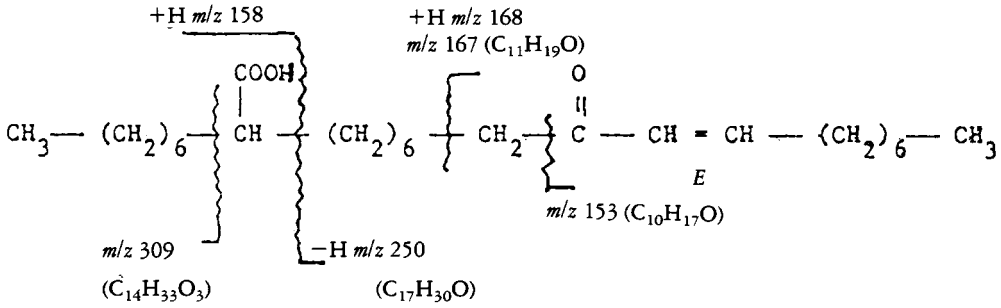
Acid **2** has a composition of $C_{28}H_{52}O_3$ (hrms), and it presents the same features as **1** in its ^{1}H-nmr and mass spectra, except for the integration of the unresolved broad band at 1.25 ppm (38H) and characteristic differences in the mass spectral fragmentation (Scheme 2).

Both compounds are apparently optically inactive, in spite of the presence of a chiral center (at C8), but this may be due to a low specific rotation coupled with a limited sample available for measurement.

Both acids were tested in vitro against L 1210 cells. Cytotoxicity was assessed by the inhibition of DNA synthesis as determined by the incorporation of ^{3}H-thymidine. Both compounds present weak, but significant, cytotoxicities with ID_{50} of 10 μg/ml for **1** and 12 μg/ml for **2**.

The small quantities of purified com-

[1]M. Guyot and M. Durgeat, unpublished data.

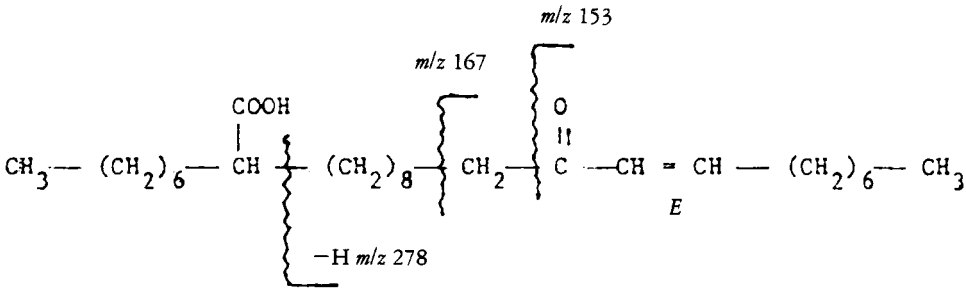


SCHEME 1

pounds isolated precluded the in vivo investigation of these acids.

The EtOH extract of a related species *Suberites inconstans* collected in the bay of Singapore (3) is cytotoxic against HeLa cells, but the active compound has not yet been isolated.

The biological properties of these two acids may be compared with those reported for related structures, such as, 10-hydroxy-2-decenoic acid (4), responsible for the activity of royal jelly against experimental leukemia and ascitic tumors, and disparolone, (*Z*)-7-oxo-10-hexadecen-1-ol (5), isolated from the gipsy moth *Porthetria dispar* L., which shows inhibitory activity against the Walker intramuscular carcinosarcoma 256 (WA) in rats.



SCHEME 2

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. The uv spectra were prepared in hexane on a Beckman Acta C 3; ir spectra were taken on a Perkin-Elmer 137 E; mass spectra were recorded on Thomson THN 208 (low resolution) and AEI MS 50 (high resolution) instruments (direct introduction, 70 eV). ^{1}H-nmr spectra were recorded in $CDCl_3$ (with TMS as internal standard) on a Bruker WP-80 spectrometer.

BIOLOGICAL MATERIAL.—*F. ficus* was collected near Roscoff, France, and identified by N. Boury-Esnault. A voucher specimen is preserved in the Laboratoire des Invertébrés Marins, Muséum National d'Histoire Naturelle, Paris.

ISOLATION OF FICULINIC ACIDS.—Fresh sponges (8 kg) were pulverized and extracted with EtOH at room temperature. After the EtOH was distilled off, the aqueous residue was extracted with hexane. The extract obtained with hexane (15 g) was separated on a silica gel column (hexane-EtOAc, 7:3, v:v), and a fraction containing acids **1** and **2** was purified by successive tlc (SiO_2 Whatman K5F, C_6H_6-EtOAc, 7:3, v:v) to yield **1** (10 mg), mp 33-35°, and **2** (7 mg), mp 31-32°; **1** and **2** were homogeneous in various systems of chromatography.

FICULINIC ACID A (**1**).—^{1}H nmr δ ppm 9.15 s, 1H, COO*H*; 6.89 dt, 1H (J=16, 6 Hz); 6.04 dt, 1H (J=16, 1.5 Hz); 2.25 m, 5H; 1.25 br s, 34H and 0.85 br t, 6H; ms *m/z* 408.359 [M^+, (9); $C_{26}H_{48}O$ requires 408.360], 390 (14), 352 (10), 349 (10), 373 (10), 295 (20), 277 (60), 250 (25), 209 (25), 195 (27), 182 (30), 140 (70), 125 (100), 60 (80).

Permanganate-periodate oxidation was performed on 2 mg of **1** (2) and led to a complex mixture in which octanoic acid was characterized as a major product by cims [$(M+H)^+$ 145].

FICULINIC ACID B (**2**).—^{1}H nmr δ ppm 9.15 s, 1H; 6.89 dt, 1H (J=16, 6 Hz); 6.04 dt, 1H (J=16, 1.5 Hz); 2.25 br s, 42H; 0.85 br t, 6H. Ir $\nu_{C=O}$ 1640, 1700 cm^{-1}; uv λ max 230 nm; ms *m/z* 436.390 [M^+, (10); $C_{28}H_{52}O_3$ requires 436.391], 408 (20), 377 (20), 319 (30), 311 (23), 305 (15), 291 (20), 278 (25), 248 (100), 203 (50), 167 (70), 153 (60), 141 (40), 60 (30).

BIOLOGICAL TESTING.—L 1210 leukemia cells (a gift from RPS-Vitry, France) were maintained in ascite form in DBA/2 mice by ip injection and cultured for 24 h, as reported (7), with various concentrations of products added in quadriplicate dose. The incorporation of ^{3}H thymidine into wells containing the products (four wells by dose) was expressed as a percentage of incorporation observed in control wells (12 wells per plate). A dose response curve was established, and the product dose corresponding to 50% value was determined. The LD_{50} is calculated from three or four representative curves.

ACKNOWLEDGMENTS

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