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N-ACYL-2-METHYLENE-β-ALANINE METHYL ESTERS FROM THE SPONGE *FASCIOSPONGIA CAVERNOSA*

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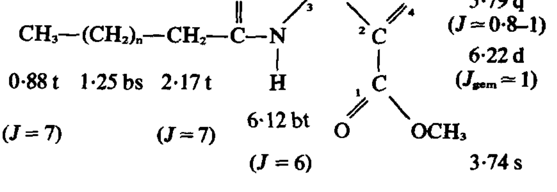
Abstract—The isolation and structural determination of a series of N-acylated 2-methylene-β-alanine methyl esters, a new naturally-occurring β-amino acid, from the sponge *Fasciospongia cavernosa*, is reported.

In the course of our investigation of natural products derived from primitive multicellular animals living in the Red Sea, we undertook the determination of the chemical compounds found in several sponges.

Fasciospongia cavernosa was found to contain a new naturally-occurring β-amino acid (**1**), which appears in the sponge in a complete series of N-acylated methyl esters, in remarkably high concentrations (between 1–2%, dry weight).

From a petrol–ether extract of the fresh dry sponge, a white material (**2**, m.p. 67°) crystallized out directly after standing at 5° for 24–48 hr. A second closely-related oily fraction (**3**) *vide infra* was obtained in a pure form only after chromatography on a silica-gel column. Compound **2** showed the presence of three functional groups in the IR spectrum: an amide ($\nu = 3260$ (bonded NH), 1640, 1550), a conjugated terminal methylene ($\nu = 940$) and a conjugated esteric group (1715, 1230 cm^{-1}). The location of these groups relative to each other in the molecule could be estimated from the following NMR spectrum data, as well as from the mass spectrum data (Scheme 1), suggesting the N-acyl-2-methylene-β-alanine methyl ester structure, shown below, for **2**:



In this NMR spectrum, the protons vicinal to the C-3-H (the latter giving rise to the signal at 4·06 dd ($J = 6$ and 0·8 Hz)) were determined by a double irradiation experiment or deuterium exchange of the NH-proton, cancelling the 6 Hz splitting; and by double irradiation of one of the terminal methylenic protons resonating at 5·79 d ($J = 0·8$ Hz). (The deuterium exchange of the amide proton could be achieved only at a relatively slow rate by DCl/D_2O or NaOD/D_2O solution, possibly because of an internal H-bond). Further evidence for the amino acid moiety of **2** could be obtained from its dihydro derivative **4**, m.p. 55° (CH_3CN), which exhibited the following NMR spectrum (Fig 1). In this spectrum the various coupling constants were confirmed by double resonance experiments and by deuterium exchange of the NH, as in the case of **2**.

According to the elemental analysis and mass spectrum (Scheme 1), it was clear that **2** was a mixture of at least five N-acyl derivatives of **1**, that is, five amides of **1** with C_{16}-C_{20} aliphatic fatty acids. In order to identify these acids, **2** was refluxed for 48 hr in saturated methanolic HCl solution until the methanolysis of the amide was complete, giving the free fatty acid methyl esters and 2-methylene-β-alanine methyl ester (**5**).

The five main fatty acid methyl esters were separated by gas chromatography and identified by their mass spectra, which were obtained by direct introduction of the compounds into the mass spectrometer. The compounds were found to be the C_{16}-C_{20} normal unbranched aliphatic carboxylic acids.

Compound **5** was characterized as the hydro chloride $C_5H_9O_2N \cdot HCl$, m.p. 93–95°. ν_{max} 3400, 2950, 2600, 1720, 980, 810 cm^{-1}; NMR (D_2O): 4·00 s (OCH_3); 4·06 s (N-CH_2); 6·29 bs and 6·73 bs

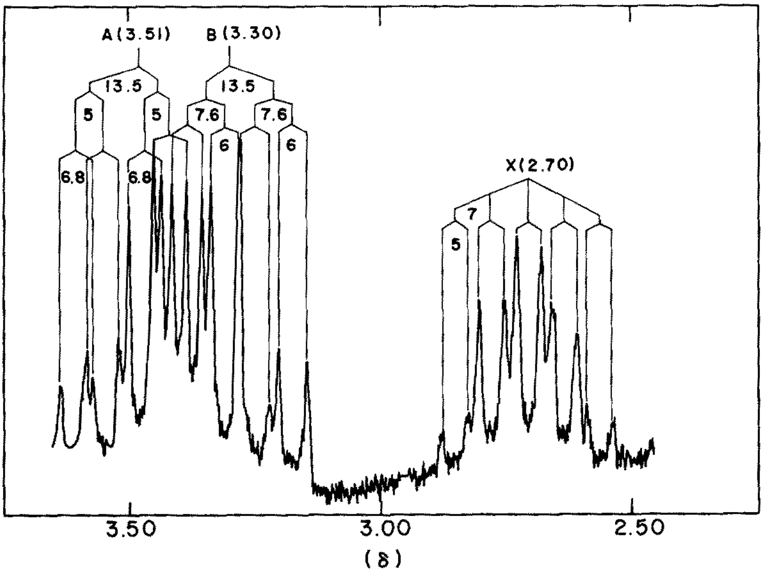
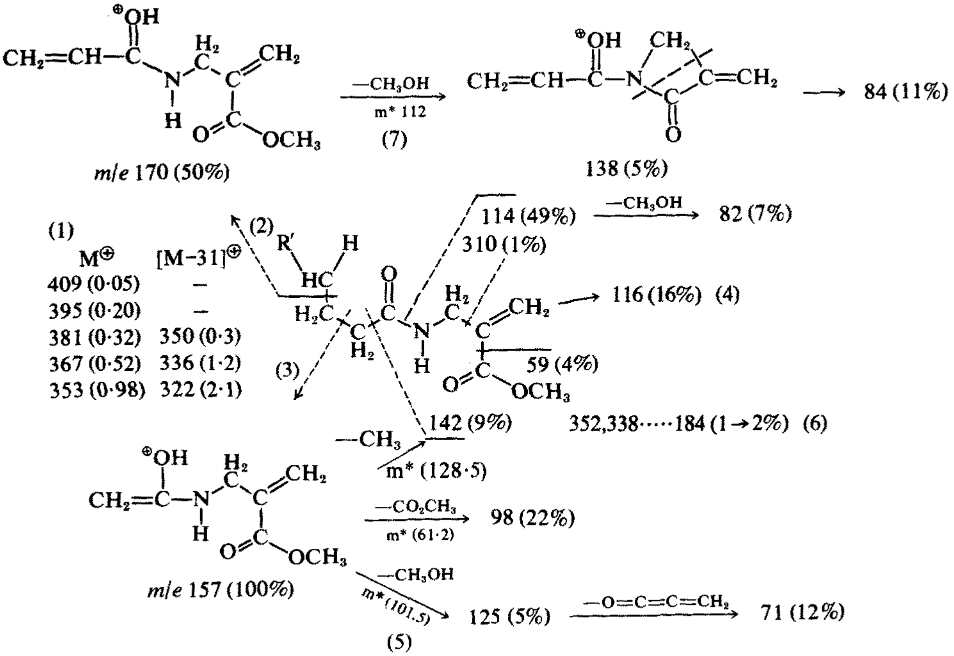
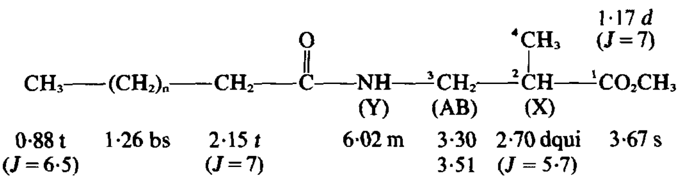


Fig 1. Partial NMR spectrum of compound **4**.



SCHEME 1. Mass spectrum of **2**.

(1) The (M—OCH₃) fragments of C_{20} and C_{19} are not strong enough to be seen.
(2) Fragmentation by a mechanism similar to that known for long-chain methyl esters.[1]
(3) A "McLafferty rearrangement".
(4) Probably the protonated amino acid methyl ester.
(5) The metastable peak was observable only in the dihydro compound.
(6) A series of fragments originating from the various esters by known fragmentations of long-chain methyl esters.
(7) Deuterium exchange (NH to ND) established this fragmentation.
(8) In the mass spectrum of **4** and N-d_1 **2**, the expected shifts according to the proposed pattern are seen.



$$CH_3—(CH_2)_n—CH_2—\overset{\overset{\displaystyle O}{\|}}{C}—\underset{(Y)}{NH}—\underset{(AB)}{{}^3CH_2}—\underset{(X)}{\overset{\overset{\displaystyle {}^4CH_3}{|}}{{}^2CH}}—{}^1CO_2CH_3$$

($J_{AB} = 13·5$; $J_{AX} = 5$; $J_{BX} = 7·6$; $J_{BY} = 6$; $J_{AY} = 6·8$ Hz)

(=CH_2); Mass spectrum: (*m/e*) 116 (38%, M+1); 115 (28%, M^+); 56 100%, $CH_2C((=CH_2)NH_2)$. Upon hydrogenation, compound **5** gave the known racemic 2-methyl-β-alanine methyl ester.[2]

Substance **3**, appearing in the amount of *ca* 1–2% (dry weight), was found to be a mixture of unsaturated, and most probably also oxygenated, fatty acids of **1**, the exact structure of which is now being studied further.

The biological activity of compounds **1–3**, which were found to cause the death of mice after subcutaneous injection in small amounts (2–4 mg per mouse, ~ 25 g wt), in comparison to other toxic amino acids,[3] is now under investigation.

EXPERIMENTAL

M.ps were taken on a Thomas Hoover capillary m.p. apparatus, and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord model 337 spectrophotometer. NMR spectra were taken on a Varian HA-100 spectrometer using 5–10% solns in $CDCl_3$, with TMS as an internal standard. Mass spectra were taken with an Hitachi Perkin-Elmer RMU 6 instrument.

Isolation of the 2-methylene-β-alanine methyl esters (**1** *and* **2**). The fresh material (300 g, dry weight) was extracted during a period of 24 hr with petrol–ether in a soxhlet. The petrol–ether extract gave, after cooling for 24 hr at 5°, a white ppt (1·5 g). The solvent was then evaporated to dryness from the remaining soln, and the crude extract obtained (16 gr) was chromatographed on a silica gel column (0·05–0·20 mm Merck 7734, 500 g) to give, after elution with petrol–ether:$CHCl_3$ (1:1), compounds **2** (0·5 g) and **3** (3·5 g). Compound **2**, m.p. 67° (after recrystallization three times from acetone–acetonitrile) had the following IR spectrum: ν_{max}^{KBr} 3260, 2880, 2810, 1715, 1640, 1550, 1460, 1430, 1290, 1260, 1190, 1150, 1110, 940, 720 cm^{-1}. (Found: C, 72·10; H, 11·12; N, 3·60. $C_{24}H_{45}O_3N$ requires: C, 72·86; H, 11·47; N, 3·54%; $C_{22}H_{41}O_3N$ requires: C, 71·88; H, 11·24; N, 3·81%).

Compound **3**, an oil, had the following IR and NMR spectra: ν_{max}^{neat} 2900, 1715, 1640, 1550 cm^{-1}; NMR ($CDCl_3$): the same as that of **2**, except for an additional broad triplet at δ 5·3 ($J = 4·5$ Hz).

Hydrogenation of **2** *to* **4**. Compound **2** (250 mg) in EtOH (25 ml) was hydrogenated over 5% Pd on $CaCO_3$ at room temp and atmospheric pressure for 18 hr. The product which was obtained following work-up was crystallized from CH_3CN (200 mg), m.p. 55°; ν_{max}^{KBr} 3300, 2900, 2830, 1735, 1640, 1550, 1470, 1180, 1130, 1110, 1050, 720 cm^{-1}; Mass spectrum: *m/e* 397 (M^+); MW calcd. for $C_{24}H_{47}O_3N$: 397 (*cf* Scheme 1).

Methanolysis of **2**. Compound **2** (500 mg) in methanolic HCl (10 ml) was refluxed for 48 hr. (After 24 hr, an additional 5 ml methanolic HCl was added). Most of the MeOH was then evaporated, ether (25 ml) was added, and the soln was extracted with water (3 × 2 ml). The aqueous soln yielded, after evaporation under reduced pressure, compound **5**, m.p. 93–95° (acetone). The fatty acid methyl esters, which were obtained from the ethereal soln, were separated by GC (15% DEGS on chromosorb W 80–100 mesh, in an 8 ft. × ¼ in. column, at 195°). They were found to have the same retention times as 16/0, 17/0, 18/0, 19/0, and 20/0 n-aliphatic methyl esters. These structures were verified by direct introduction of the esters into the mass spectrometer.

Hydrogenation of **5** *to* (±)*β-methyl alanine methyl ester hydrochloride.*

Compound **5** (20 mg) in EtOH (20 ml) was hydrogenated over 5% Pd on charcoal at room temp and atmospheric pressure for 12 hr. Following work-up, the (±)methyl β-amino-α-methylpropionate (5 mg), m.p. 108° (lit.[2] 110°) was isolated.

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