THE ISOLATION OF α-METHYLENE-β-ALANINE, A HERBICIDAL MICROBIAL METABOLITE

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In the course of screening for herbicidal substances produced by microorganisms[1)], we have isolated a metabolite of an unclassified *Streptomyces* sp. A12701[††] which inhibits the growth of *Arabidopsis thaliana*, and established its structure to be α-methylene-β-alanine (**1**). While **1** has not previously been reported to be a microbial natural product, KASHMAN *et al.*[2)], and YUNKER and SCHEUER[3)] have reported its isolation from the Red Sea sponge *Fasciospongia cavernosa* and the black Hawaiian sponge *Spongia* cf. *zimocca*, respectively, as a family of *N*-acylated amino acid methyl esters. HOLM and SCHEUER[4)] subsequently reported the synthesis of α-methylene-β-alanine as the hydrochloride salt. This paper describes the production, isolation, physico-chemical properties, structure, and herbicidal activity of this novel microbial metabolite.

Streptomyces sp. A12701 was isolated from a soil sample collected at Beach Pond State Park in Rhode Island, U.S.A. A 250-ml Erlenmeyer flask containing 50 ml of a medium consisting of Tryptone 0.5% and yeast extract 0.3% was inoculated and fermented at 30°C for 3 days on a rotary shaker at 250 rpm. Fifty ml of the inoculum broth was transferred to a 2-liter Erlenmeyer flask containing 1 liter of production medium consisting of dextrin 1.0%, glucose 0.1%, soybean flour 0.5%, yeast extract 0.15%, and $CaCO_3$ 0.15% (pH 6.5). The fermentation was carried out on a rotary shaker at 30°C and 120 rpm for 4 days. The herbicidal activity of the resulting broth was assayed by an agar diffusion method using *A. thaliana* as the test organism.

The herbicidal metabolite produced by A12701 was extracted from 550 ml of sterile culture filtrate by adsorption onto Bio-Rad AG50W-X8 cation exchange resin. Elution with 3% NH_4OH gave 0.35 g of crude, active material which was fractionated further by RP C18 flash chromatography using water as the eluting solvent. The active fractions (0.18 g) were combined, evaporated under reduced pressure, and further purified on two successive Sephadex LH-20 columns using water - methanol (3 : 1) and water - methanol (1 : 3) elution to yield 0.015 g of high purity α-methylene-β-alanine (**1**).

α-Methylene-β-alanine is readily soluble in water and methanol. The molecular formula was established as $C_4H_7NO_2$ by HRFAB-MS ($(M+H)^+$ m/z 102.0545, Calcd: 102.0555). The UV spectrum of **1** in water showed only end absorption. The IR spectrum (neat) contained bands at 1650 cm^{-1} assigned to a carbon-carbon double bond and at 1560 and 1380 cm^{-1} for a carboxylate salt, thus accounting for the two unsaturations in the molecule. The 300 MHz ^1H NMR spectrum in D_2O showed two uncoupled protons at δ 6.03 (s) and δ 5.64 (s), suggestive of a geminally disubstituted olefin. The remaining feature in the ^1H NMR spectrum of **1** was a two proton singlet at δ 3.67. The three unobserved protons were attributed to the exchangeable protons of carboxylic acid and primary amine groups. Compound **1** gave a positive color reaction upon treatment with ninhydrin. Each of the four carbons present in **1** were observed in the 75 MHz ^{13}C NMR spectrum (δ 170.76, 135.43, 124.38, and 39.72).

Consideration of this data enabled the establishment of two possible structures: α-methylene-β-alanine and β-methylene-β-alanine. Based upon the downfield chemical shifts of the olefinic proton signals, the olefinic carbon chemical shifts, and the expected instability of β-methylene-β-alanine, the structure of the herbicidal substance produced by A12701 was concluded to be α-methylene-β-alanine (**1**). An agar-based titration study against *A. thaliana* showed that **1** caused growth reduction and slight chlorosis at 50 μg/ml, while higher rates resulted in complete inhibition of germination. In small scale post-emergence applications, α-methylene-β-alanine showed weak activity on velvetleaf at 5.6 kg/ha.

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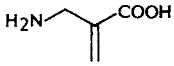

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Although the above spectral data strongly supported the identification of the A12701 herbicidal metabolite as α-methylene-β-alanine, the proton chemical shifts observed for this compound were consistently upfield of those for the synthetically-derived hydrochloride salt by 0.6~0.9 ppm[4]. Conversion of 2 mg of **1** to its hydrochloride salt with 1 N HCl resulted in excellent agreement of proton chemical shifts (300 MHz, D_2O) when the $N\text{-}CH_2$ resonance was referenced to the corresponding literature value (δ 4.25) for the synthetic material.

The identification of a naturally occurring herbicidal amino acid, α-methylene-β-alanine, has been reported. While this compound has been cited as a constituent of several sponge metabolites, it has not previously been reported to occur in its free form in nature and its herbicidal properties were previously unknown.

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References

1) Ayer, S. W.; B. G. Isaac, D. M. Krupa, K. E. Crosby, L. J. Letendre & R. J. Stonard: Herbicidal compounds from micro-organisms. Pestic. Sci. 27: 221~223, 1989
2) Kashman, Y.; L. Fishelson & I. Ne'eman: *N*-Acyl-2-methylene-β-alanine methyl esters from the sponge *Fasciospongia cavernosa*. Tetrahedron 29: 3655~3657, 1973
3) Yunker, M. B. & P. J. Scheuer: Alpha-oxygenated fatty acids occurring as amides of 2-methylene-β-alanine in a marine sponge. Tetrahedron Lett. 1978: 4651~4652, 1978
4) Holm, A. & P. J. Scheuer: Synthesis of α-methylene-β-alanine and one of its naturally occurring α-ketoamides. Tetrahedron Lett. 21: 1125~1128, 1980