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NEW ANTIMETABOLITE ANTIBIOTICS RELATED TO BIOTIN: α-METHYLDETHIOBIOTIN AND α-METHYLBIOTIN (1)

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The isolation of two new antimetabolite antibiotics, α-methyldethiobiotin (αMDB) and α-methylbiotin (αMB), from fermentation of a strain of *Streptomyces lydicus* is herein described; since αMB was formed in only trace amounts, a synthesis of the racemic antibiotic was developed to permit biological evaluation.

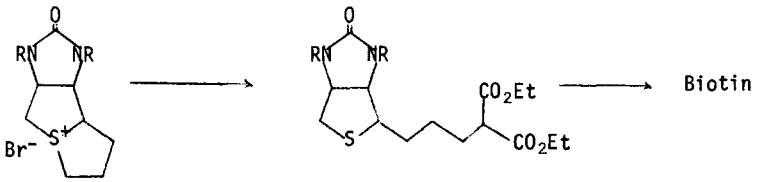
Although bioautography of crude α-dehydrobiotin (αDB) (2) isolated from fermentation of a strain of *S. lydicus* on *Saccharomyces pastorianus* indicated that the antibiotic was homogeneous, bioautography on *Bacillus subtilis* grown in a synthetic medium (3) demonstrated the presence of two significant activities in addition to αDB. These activities were slightly less polar than αDB and were similarly reversed by the presence of biotin (B). Subsequent work established that these additional antimetabolite antibiotics were present in small amounts but were considerably more potent *vs. B. subtilis* (synthetic medium) than αDB.

The least polar antibiotic, easily separated from crude αDB by virtue of its greater solubility in organic solvents, was isolated from aqueous acetone fractions eluted from carbon prior to the fractions containing the bulk of the αDB (2). The aqueous concentrate of these fractions was readsorbed on carbon and eluted with aqueous acetone. Prolonged cooling of the concentrated eluate afforded the antibiotic as a crude solid. Several recrystallizations (acetone) afforded the pure antibiotic, mp 161.5-162.5°. High-resolution mass spectrometry established that the antimetabolite had the composition $C_{11}H_{20}N_2O_3$ corresponding to dethiobiotin (DB) plus a CH_3 group. Comparison of the antibiotic's NMR spectrum (d_7DMF) with that of DB indicated that it was αMDB; the additional CH_3 appeared as a doublet (J = 7 Hz) at δ1.12 ppm and only one proton, observed as a complex multiplet, was adjacent to the CO_2H rather than the two-proton distorted triplet of DB. A plain negative ORD curve similar to DB (4) was observed

for αMDB indicating that the DB nucleus probably had the configuration of natural DB; the steric configuration of the asymmetric α carbon has not been determined.

The other new antibiotic resisted separation from αDB until resolution of their phenacyl esters was achieved. Repeated recrystallization of the phenacyl ester of crude αDB (containing the ester of the new antibiotic) led to an excellent recovery of pure αDB phenacyl ester, mp 175.177°, from which pure αDB, mp 251.5-252° (5), was obtained by mild alkaline hydrolysis. Partition chromatography of mother liquor residues afforded a mixture of the phenacyl esters of the new antibiotic and αMDB which was resolved on silica gel. High-resolution mass spectrometry established the composition of the new antibiotic's phenacyl ester as $C_{19}H_{24}N_2O_4S$ consistent with B phenacyl ester plus one CH_3 group. In its NMR spectrum ($CDCl_3$), the CH_3 appeared at δ1.25 ppm (d, J = 6 Hz), reasonable for a side chain CH_3; the apparent absence of protons adjacent to the ester function, clearly evident as a distorted triplet in the δ2.5 ppm region of the spectrum of B phenacyl ester, indicated that the unknown phenacyl ester was most likely that of αMB. A positive Cotton effect, similar to that observed for B phenacyl ester, was observed in the ORD curve of αMB phenacyl ester indicating that the B nucleus probably had the configuration of natural B; the configuration of the asymmetric α carbon has not been determined. Bioautography established the identity of the αMB, after alkaline hydrolysis of the phenacyl ester, with the original contaminating activity in the crude αDB sample.

Since fermentation titers were extremely low, a synthesis of αMB was desired to permit biological evaluation. In a Hoffmann-LaRoche synthesis of B (6), racemic N-blocked thiophanium salt 1 was reacted with the Na salt of diethylmalonate in excess diethylmalonate at 150°, affording malonic ester 2. Racemic B was then obtained after hydrolysis, decarboxylation, and debenzylation.



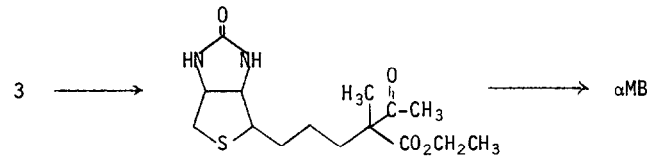
1 R = $\emptyset CH_2$-

2 R = $\emptyset CH_2$-

3 R = H

However, when the racemic unblocked thiophanium salt 3 (6b, 7) was reacted under similar conditions with diethyl methylmalonate anion, the desired transformation to the malonic ester precursor of αMB was complicated by N-ethylation and other side reactions. After hydrolysis and decarboxylation, extensive purification was required to obtain a modest yield of crystalline racemic αMB.

A far superior conversion of 3 into racemic αMB was realized with the Tl salt of ethyl methylacetoacetate. Ethyl acetoacetate was methylated *via* its Tl salt (8) and the methylated derivative converted into its crystalline Tl salt which was found to slowly but smoothly react with a DMF suspension of thiophanium salt 3 at room temperature, affording the acetyl precursor of αMB 4. Acetyl ester 4 was isolated in almost quantitative yield by chromatography as a TLC homogeneous oil [mass spectrum M^+ at 328; NMR ($CDCl_3$) δ1.33 ppm (s, 3, $-CH_3$), δ2.15 ppm (s, 3, $CH_3-\overset{O}{\overset{\|}{C}}-$)]. Alcoholysis of the acetyl group (9) followed by hydrolysis of the ester afforded crystalline racemic αMB, mp 183-186°, upon acidification. The over-all yield from sulfonium salt 3 to αMB was 72%. Recrystallization afforded analytically pure αMB [mp 186-188°; anal. ($C_{11}H_{18}N_2O_3S$) C, H, N, S; NMR (d_7DMF) δ1.12 ppm (d, 3, J = 7 Hz; $-CH_3$)]. Bioautography established that the synthetic racemic αMB had the same paper strip mobility as the antibiotic from fermentation; moreover, the NMR spectrum of the phenacyl ester obtained from the synthetic sample was the same as that of the phenacyl ester of the natural product.



4

Biological evaluation of the biotin antimetabolites is in progress; αDB, αMB, and αMDB have all shown highly specific *in vitro* activity against members of the genus *Mycobacterium* (1). The *in vitro* activity of αMDB against this group of microorganisms was comparable to that of streptomycin sulfate (10).

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