Synthesis of an Analogue of Mycobactin

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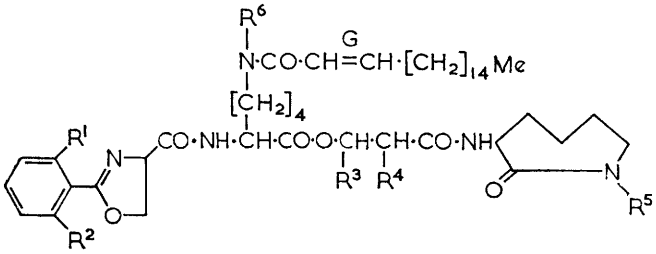
An analogue (III) of the mycobacterial growth factor mycobactin has been synthesised. The analogue lacks the two hydroxamic acid linkages and the phenolic hydroxy-group necessary for chelation of iron(III).

MYCOBACTIN is a growth factor which chelates iron(III) and which stimulates the growth of mycobacteria. The structure of one of the mycobatins (I) was put forward in 1954 by Snow.[1] Later, the factor was named mycobactin P to distinguish it from closely related substances and a more detailed structure, indicating the appropriate stereochemistry was put forward for the major component.[2] The compound was isolated from *Mycobacterium phlei* and was shown to stimulate the growth of *M. johnei* at very low concentrations.

More recently, mycobactin T (II) was isolated from *M. tuberculosis;* this factor also promotes the growth of *M. johnei*.[3]

As part of a programme aimed at evolving new antituberculosis agents, we have synthesised the analogue (III), a compound related to the mycobactins but lacking the groups necessary to complex with iron(II).



	R¹	R²	R³	R⁴	R⁵	R⁶	G
(I)	OH	CH_3	Et	Me	OH	OH	*cis*
(II)	OH	H	Me	H	OH	OH	*cis*
(III)	H	H	Me	H	H	H	*trans*

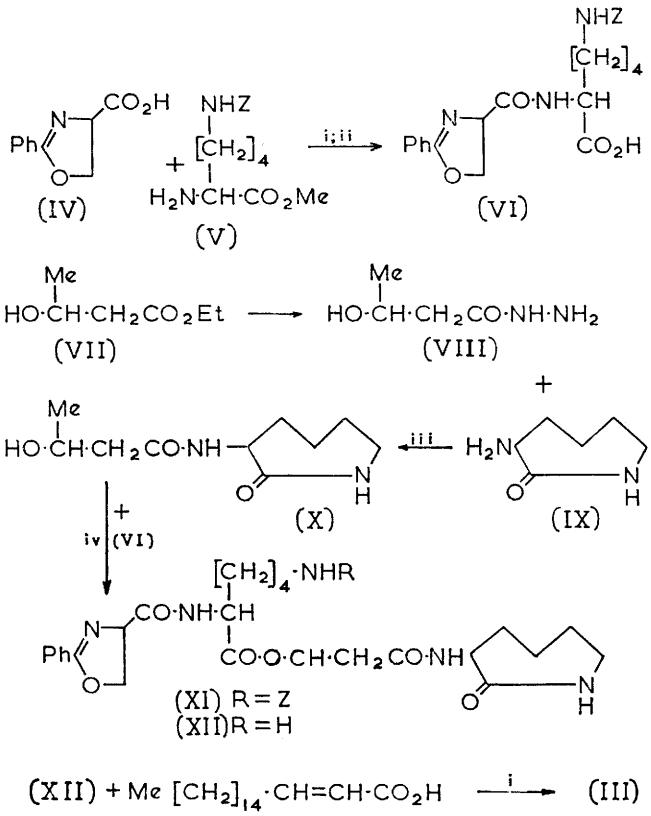
The route that was finally evolved entailed the synthesis of units (VI) and (X), formation of the ester bond between them and attachment of the lipophilic C_{18} side-chain.

Compound (VI) was prepared by linking DL-2-phenyl-2-oxazoline-4-carboxylic acid (IV) with the protected lysine ester (V) in the presence of dicyclohexylcarbodiimide (DCCI) and saponifying the product.

The hexanolactam derivative (X) is analogous to the mycobactin degradation product cobactin, a cyclic hydroxamic acid, and was synthesised from DL-3-aminohexanolactam and DL-3-hydroxybutyric acid hydrazide by the azide method.

Several methods were tried to link (VI) and (X). The use of *NN'*-carbonyldi-imidazole gave the best results, although Shemyakin's method also afforded the required ester.[4] After hydrogenolysis of the benzyloxycarbonyl group, the final step, the reaction of (XIII) with *trans*-octadec-2-enoic acid, was achieved by using DCCI. Better yields were obtained by treating the hydrogenolysis product with the octadecenoic acid chloride.

The n.m.r. spectrum of (III) was complex, but it was possible to distinguish the vinyl protons of the *trans*-double-bond, the characteristic pattern associated with



SCHEME

Z = Benzyloxycarbonyl

Reagents: i, dicyclohexylcarbodi-imide; ii, OH^-; iii, HNO_2; iv, *NN'*-carbonyldi-imidazole

the three oxazoline protons, the hydrogen atoms of the phenyl group, and the doublet expected for the methyl group of the butyric acid fragment.

It was possible to detect the parent ion in the mass spectrum of (III); accurate mass measurement confirmed that the mycobactin analogue had the required molecular formula $C_{44}H_{69}N_5O_7$. The compound showed no significent activity against *M. tuberculosis* H37Rv in mice.

EXPERIMENTAL

6-Benzyloxycarbonylamino-2(2-phenyl-2-oxazoline-4-carboxamido)hexanoic Acid (VI).—N^6-Benzyloxycarbonyl-L-lys-

[1] G. A. Snow, *J. Chem. Soc.*, 1954, 2588, 4080.
[2] G. A. Snow, *Biochem. J.*, 1965, **94**, 160.
[3] G. A. Snow, *Biochem. J.*, 1965, **97**, 166.
[4] M. M. Shemyakin, *Angew. Chem.*, 1960, **72**, 342.

ine methyl ester hydrochloride (18·7 g., 0·055 mole) was dissolved in aqueous 10% sodium carbonate (100 ml.) and the solution was extracted several times with cold ethyl acetate. The combined extracts were dried briefly ($MgSO_4$) and 2-phenyl-2-oxazoline-4-carboxylic acid (9·6 g., 0·05 mole) and dicyclohexylcarbodi-imide (10·3 g., 0·05 mole) were added to the solution. The mixture was shaken and kept at room temperature overnight. The precipitated dicyclohexylurea was filtered off and the solution was washed, successively, with N-hydrochloric acid, aqueous 10% sodium carbonate, and water; it was then dried ($MgSO_4$). The solvent was removed and the residue was washed with light petroleum (b.p. 40—60°).

The viscous syrup (22·0 g., 0·047 mole) was dissolved in methanol (170 ml.) and aqueous N-sodium hydroxide (55 ml.) was added. The solution was stirred at room temperature for 4 hr. and then water (1·5 l.) was added. Methanol was removed under reduced pressure and the solution was then washed with ether and filtered. The acid product (15·6 g., 72%) precipitated by the addition of N-hydrochloric acid (55 ml.) was collected and dried. A sample had m.p. 165—167° (from ethyl acetate) (Found: C, 63·4; H, 6·15; N, 9·2. $C_{24}H_{27}N_3O_6$ requires C, 63·6; H, 6·0; N, 9·3%) $\nu_{max.}$ (Nujol) 3400 (OH), 3300 (NH), 1680, 1660, 1630, 1560, and 1530 cm^{-1}., $\lambda_{max.}$ (MeOH) 245 (log ε 4·06); τ ($CDCl_3$) 8·0—8·8 (6H), 6·9 (2H), 5·0—5·5 (4H), 4·9 (2H), and 2·6 (10H).

3-(3-*Hydroxybutanamido*)*hexahydro*-2H-*azepin*-2-*one* (X).—A solution of sodium nitrite (4·0 g., 0·058 mole) in water (15 ml.) was added with stirring to a cooled solution of 3-hydroxybutyric acid hydrazide (5·0 g., 0·042 mole) in N-hydrochloric acid (42·5 ml.). After a few minutes, a solution of 3-amino-hexanolactam (5·4 g., 0·042 mole) in water (15 ml.) was added. The solution was stirred on 0° for 1 hr. and stored at room temperature overnight. The solution was then continuously extracted with chloroform. The extract was dried and evaporated and the residue was crystallised from ethyl acetate to give the *amide* (3·2 g., 35%) as fine white crystals, m.p. 183—185° (Found: C, 56·1; H, 8·2; N, 12·8. $C_{10}H_{18}N_2O_3$ requires C, 56·0; H, 8·5; N, 13·0%) $\nu_{max.}$ 3450 (OH), 3250 (NH), 1620, and 1660 (C=O) cm^{-1}., τ (D_2O) 8·7 (3H), 7·9—8·5 (6H), 7·5 (2H) 6·6 (2H), and 5·7 (1H).

Ester (XI).—A solution of the carboxylic acid (VI) (0·9 g., 0·002 mole) in dry tetrahydrofuran (20 ml.) was heated under reflux for 2 hr. with *NN'*-carbonyldi-imidazole (0·96 g., 0·006 mole). The hydroxyamide (X) (1·28 g., 0·006 mole) was added and the solution was heated under reflux for a further 3 hr. The solvent was removed under reduced pressure, and the product taken up in ethyl acetate and washed twice with water. The solution was dried ($MgSO_4$) and evaporated to yield a clear gum. This material was purified by stirring it under water, dissolving it in aqueous methanol, and filtering the solution through a column of IR-45 ion-exchange resin. The solvent was removed from the filtrate and the residue was dried *in vacuo* to yield the *ester* (0·5 g., 40%) as a hygroscopic solid (Found: C, 62·4; H, 7·2; N, 11·1. $C_{34}H_{43}N_5O_8$ requires C, 62·8; H, 6·7; N, 10·8%).

Mycobactin Analogue (III).—(*a*) The ester (XI) (1·4 g., 0·0022 mole) was treated with hydrogen in methanol over 10% palladized charcoal. When evolution of carbon dioxide had ceased, the solution was filtered and evaporated. The residual gum was taken up in redistilled methylene chloride (20 ml.) and the solution was cooled to 0°. *trans*-2-Octadecenoic acid (0·56 g., 0·002 mole) and dicyclohexylcarbodi-imide (0·41 g., 0·002 mole) were added and the solution shaken for several hours and kept overnight. The dicyclohexylurea was filtered off and the solvent was evaporated. The crude product was washed with light petroleum (b.p. 40—60°) and precipitated from methanol with water. The compound was further purified by chromatography on silica gel with chloroform–methanol. The *analogue* (50 mg., 3%) was obtained as a white amorphous powder, m.p. 156—160°. The compound showed only one spot on two t.l.c. systems (Found: C, 67·6; H, 8·6; N, 9·0. $C_{44}H_{69}N_5O_7$ requires C, 67·8; H, 8·9; N, 9·0%), $\nu_{max.}$ 3300 (NH), 1720, 1640, and 1530 cm^{-1}.

(*b*) The ester (XI) (0·7 g., 0·0011 mole) was hydrogenolyzed as before. After removal of the solvent the residual oil was taken up in dry chloroform and treated with the acid chloride of *trans*-octadec-2-enoic acid [(prepared from acid (0·3 g.) with thionyl chloride] in dry chloroform while being cooled and with the simultaneous addition of triethylamine (0·5 ml.). The solution was stirred for 2 hr. at 0° and then overnight at room temperature. The solution was washed with water, 5% sodium hydrogen carbonate, and water; it was then dried. Removal of the solvent gave an oily product which was purified as before to give the *analogue* (300 mg., 35%) identical with the material obtained by the other method.

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