NEW PLEUROMUTILIN DERIVATIVES WITH ENHANCED ANTIMICROBIAL ACTIVITY

I. SYNTHESIS

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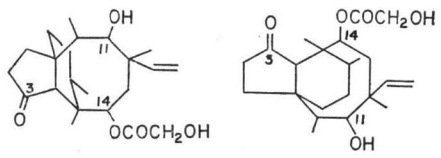
A series of new derivatives of the antibiotic pleuromutilin, produced by some Basidiomycetes, was synthesized by chemical modification of natural pleuromutilin. Most of them contain basic functional groups in the side chain at C_{14} of the mutilin skeleton. The monotosylate of pleuromutilin was used as a versatile intermediate for displacement by N-, O- and S-nucleophiles.

In 1951 KAVANAGH and coworkers[1] described the isolation and characterization of a crystalline antibiotic substance from two species of the Basidiomycete genus *Pleurotus*, which they named pleuromutilin. They proposed the empirical formula $C_{22}H_{34}O_5$ which was later confirmed, and observed high antimicrobial activity, principally against Gram-positive bacteria *in vitro*. Further chemical characterization was undertaken by ANCHEL.[2] A decade later ARIGONI and his group in Zürich[3,4] established the diterpene structure and elucidated the biosynthetic pathway to a considerable extent[5,6,7]. BIRCH and coworkers in Manchester came independently to the same conclusions[8,9].

The structure of pleuromutilin (**1**) can be drawn in several different ways, two of which are shown in Chart 1. For the sake of simplicity only the second of these will be used in this paper, as stereochemical problems will not be dealt with.

These formulae show the tricyclic carbon skeleton of pleuromutilin, of which the eight-membered ring is the most characteristic feature. Pleuromutilin has a glycolate ester, a carbonyl group, a secondary OH-group and a vinyl group as functional groups. The bis-secondary diol formed by alkaline hydrolysis of pleuromutilin is known as mutilin[8].

Chart 1. Structure of pleuromutilin



In 1963 a group under BRANDL at the Biochemie Ges.m.b.H. isolated an antibiotic from *Clitopilus passeckerianus* that they showed to be identical with pleuromutilin[10,11]. Some years later inhibition of the growth of mycoplasmas by pleuromutilin was observed, a property of interest because the importance of mycoplasmas as pathogens in man and domestic animals has been increasingly recognized in recent years[12,13].

Attempts were therefore made at Biochemie Ges.m.b.H. to prepare derivatives suitable for chemotherapeutic application.[10,14,15]

Some years afterwards we began a systematic study of structural variations and their correlation with changes observed in antimicrobial activity. This enabled us to recognize the structure-activity relationships, discussed in the following paper[16]. From these studies resulted derivatives with greatly

improved antibiotic activity and of potential value in the chemotherapy of infections caused by mycoplasma and Gram-positive bacteria.

Natural pleuromutilin obtained by fermentation[11] served as starting material for all compounds synthesized.

In the course of extensive degradative studies of pleuromutilin by ARIGONI[3~6], BIRCH[8,9] and their respective coworkers a large number of transformation products were prepared. These exhaustive chemical studies paved the way for further selective modifications. Synthesis of derivatives necessarily focusses attention on the functional groups of pleuromutilin, the principal characteristics of which are summarized below.[3~6,8,9]

The carbonyl group of the five-membered ring is sterically hindered, but affords an oxime **60** under forcing conditions, undergoes HUANG-MINLON reduction to methylene and reduction with $NaBH_4$ to the hydroxy group. The vinyl group can easily be hydrogenated. The glycolate ester undergoes smooth alkaline hydrolysis yielding mutilin. This places a restraint on the choice of reaction conditions in the synthesis of derivatives but is not paralleled by any marked sensitivity to hydrolysis under biological conditions.

The two secondary hydroxyl groups of mutilin differ in reactivity. The 14-OH group, which in pleuromutilin occurs esterified with glycolic acid is in general more difficult to acylate than the 11-OH group. A consequence is that O-acylation of mutilin yields mixtures in which the antimicrobially inactive 11-O-acyl derivative usually predominates. Preparation of 14-O-acyl derivatives necessitates either selective hydrolysis of 11,14-disubstituted derivatives (*e. g.* preparation of the 14-O-acetate) or separation of isomers. Compounds **49**, **50** and **51** were synthesized by the latter method. Acylation of mutilin with 5-dimethyl aminohexanoyl chloride can be made to yield predominantly either the monoacyl derivatives **49** and **50** or the diacyl derivative **51**. Analysis of the reaction mixtures by ^{1}H-NMR showed that the 11- and 14-mono-O-acyl derivatives in this case were formed in approximately equal amounts by either method, monoacylation or cautious hydrolysis of the diacyl compound **51**. Separation was achieved on TLC plates impregnated with $AgNO_3$, a method which naturally failed to separate mixtures of the dihydro analogues.

Transanular hydride shifts within the eight-membered ring seriously limit the range of reactions that are possible at C-11 without skeletal modification[8]. Synthesis of derivatives of the 11-oxo group was unsuccessful. Oxidation of the C_{11}-OH smoothly forms the 11-oxo compounds.

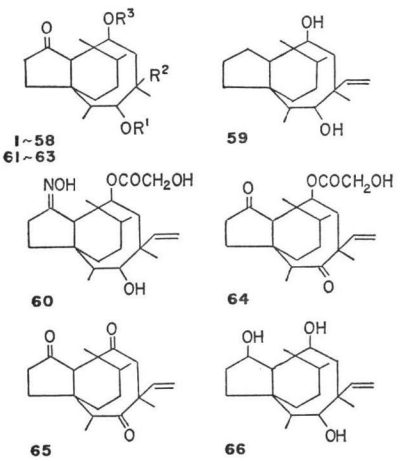
The most convenient and versatile approach to the problem of modifying pleuromutilin proved to be by way of the primary hydroxy group, which readily undergoes selective esterification, a fact previously exploited for the synthesis of derivatives with carboxyl groups in the side chain[10]. Sulfonate esters **3**, **4**, **5** were especially useful, in particular the tosylate, which crystallizes readily. RIEDL prepared a series of derivatives by displacement of tosylate with various nucleophiles[15]. Preparation of the aromatic derivatives **36**~**39** with a free phenolic OH-group was achieved in this way by reaction with the corresponding thiophenols. The products were then alkylated by us with various dialkylaminoalkylhalides, the yields in this latter step were moderate and variable. We found xylene/aqueous potassium carbonate to be a fairly suitable system.

Displacement of tosylate in **3** by O-nucleophiles, for example by diethylaminoaminoethanol to yield **11**, also gives generally poor results, hydrolysis to mutilin with substitution competing or even predominating. Substitution by primary or secondary amines is more facile, (**9**, **10** and **6**, **7**, **8**), while

reaction with aliphatic thiols, bearing basic substituents, as with thiophenols, proceeds especially smoothly to yield the derivatives **12** to **35**. This series was varied systematically on account of the high antimicrobial activity of its members[16]. The necessary thiols were prepared by standard methods, usually from the corresponding aminoalcohols via the chloride-hydrochloride and isothiouronium salt. Addition of secondary amines to ethylene sulphide proved to be a good general method for preparing the dialkylaminoethanethiols[17].

Basic diesters were made by selective acylation of the primary hydroxy group of pleuromutilin with acid chlorides of the type $ClCO(CH_2)_nNR_2$ in DMF. During work-up the lower homologues (n= 1 and 2) are so rapidly hydrolyzed to pleuromutilin that neither chromatographic nor extractive isolation was possible. Compound **47** is, however, stable to such a degree that salt formation with fumaric acid is possible with no more than minimal hydrolysis.

Chart 2. Derivatives of pleuromutilin



Experimental

Melting points were determined on a Kofler hot-stage microscope (Reichert). NMR spectra were recorded on a Varian HA 100 spectrometer, IR spectra on a Perkin-Elmer spectrophotometer 421. Spectral features were in full accord with structures. Analytical results of the new compounds listed in the Tables were within 0.4% of the theoretical values.

The TLC were developed on Silica Gel (Merck "Fertigplatten") and the spots made visible with a solution of 2% $Ce(SO_4)_2$ in 10% aqueous sulfuric acid after heating at 120°C. Column chromatography was done on Silica Gel ("Kieselgel 60" Merck, 230~400 mesh).

Method A:

Pleuromutilin, 0.38 g (1.0 m mole), was dissolved in 1.5 ml of chloroform, the solution cooled to 0°C and 0.22 ml (1.5 m mole) of triethylamine added followed by 0.11 ml (1.3 m mole) of methanesulfonyl chloride. The reaction mixture was stirred for 5 hours at 20°C, washed three times with water, evaporated and crystallized from ethylacetate-ether to give 0.37 g (81%) of **5** as colorless crystals melting at 149~150°C, homogeneous on TLC (benzene - ethylacetate, 2: 1).

Method B:

A solution of 5.33 g (10 m mole) of 14-deoxy-14-tosyloxy-acetoxy mutilin **3** and 3 ml (28.8 m mole) of diethylamine in 30 ml of absolute ethanol was refluxed for 4 hours. After removal of the solvent under reduced pressure, the residue was dissolved in dichlormethane (30 ml), washed twice with water and concentrated to half of its volume. A solution of 1.16 g (10 m mole) of maleic acid in 12 ml of methanol was added. Finally the mixture was gradually diluted with ether (200 ml). On standing overnight at room temperature the salt crystallized. Compound **6** was obtained as the hydrogenmaleate melting at 175~177°C (3.83 g, 70% yield). Purity was checked by TLC with benzene - ethylacetate (2: 1), in the cases of the diamines **8**, **9** and **10** using chloroform - methanol (1: 2).

Method C:

One gram (23 m mole) of a 55% dispersion of sodium hydride in oil was suspended in 15 ml of absolute dimethylformamide. To this mixture 2.60 g (22 m mole) of diethylaminoethanol (distilled from KOH) were dropped with stirring at room temperature. After hydrogen evolution had ceased, the reaction mixture was cooled to −11°C. A solution of 10.7 g (20 m mole) of 14-deoxy-14-tosyloxy-acetoxy mutilin in 10 ml of absolute dimethylformamide was added drop by drop. The resulting mix-

Table 1. Pleuromutilin derivatives **1**~**66**

No.	R^1	R^2	R^3	Method of synthesis	Mp, °C (Salt)
1	H	vinyl	$COCH_2OH$	Lit.[1,3,9]	167~168
2	H	Et	$COCH_2OH$	Lit.[3,9]	150.5
3	H	vinyl	$COCH_2OSO_2C_6H_4$-4-Me	Lit.[15]	147.5~149.5
4	H	Et	$COCH_2OSO_2C_6H_4$-4-Me	A	78~80[a)]
5	H	vinyl	$COCH_2OSO_2CH_3$	A	149~150
6	H	vinyl	$COCH_2NEt_2$	B	175~177 (hml[b)])
7	H	vinyl	$COCH_2$-morpholino	B	195~200 (ch[e)])
8	H	vinyl	$COCH_2$-piperazino-$(CH_2)_2OH$	B	133~137 (dch[d)])
9	H	vinyl	$COCH_2NH(CH_2)_2NEt_2$	B	150[a)] (dch)
10	H	vinyl	$COCH_2NH(CH_2)_3$-morpholino	B	190[a)] (dch)
11	H	vinyl	$COCH_2O(CH_2)_2NEt_2$	C	80[a)] (hfu[e)])
12	H	vinyl	$COCH_2S(CH_2)_2NMe_2$	D	95[a)] (ch)
13	H	vinyl	$COCH_2S(CH_2)_2NEt_2$	D	147~148 (hfu)
14	H	vinyl	$COCH_2S(CH_2)_2N(i\text{-}Pr)_2$	D	200[a)] (ch)
15	H	vinyl	$COCH_2S(CH_2)_2N(n\text{-}Bu)_2$	D	90[a)] (ch)
16	H	vinyl	$COCH_2S(CH_2)_2N(2\text{-}Et\text{-}hexyl)_2$	D	62[a)] (ch)
17	H	vinyl	$COCH_2S(CH_2)_3NMe_2$	D	120~125 (ch)
18	H	vinyl	$COCH_2S(CH_2)_3N(n\text{-}Bu)_2$	D	50[a)] (ch)
19	H	vinyl	$COCH_2S(CH_2)_5NMe_2$	D	80[a)] (hfu)
20	H	Et	$COCH_2S(CH_2)_2NMe_2$	D	110[a)] (ch)
21	H	Et	$COCH_2S(CH_2)_2NEt_2$	D	100 (hfu)
22	H	Et	$COCH_2S(CH_2)_2N(n\text{-}Bu)_2$	D	90[a)] (ch)
23	H	vinyl	$COCH_2S(CH_2)_2$-pyrrolidino	D	100[a)] (ch)
24	H	vinyl	$COCH_2S(CH_2)_2$-piperidino	D	90[a)] (ch)
25	H	vinyl	$COCH_2S(CH_2)_2$-hexahydroazepino	D	120[a)] (ch)
26	H	vinyl	$COCH_2S(CH_2)_2$-morpholino	D	128[a)] (ch)
27	H	vinyl	$COCH_2S(CH_2)_2$-thiomorpholino	D	125[a)] (ch)
28	H	vinyl	$COCH_2S(CH_2)_2$-piperazino-Me	D	150~154(dch), 160~162(zml[f)])
29	H	vinyl	$COCH_2S(CH_2)_2$-piperazino-$(CH_2)_2OH$	D	135~140(dch), 137~139(zml)
30	H	vinyl	$COCH_2S(CH_2)_2$-piperazino-$(CH_2)_2OAc$	D	127~131(dch), 142~144(zml)
31	H	vinyl	$COCH_2S(CH_2)_2$-piperazino-n-Bu	D	165~167 (zml)
32	H	vinyl	$COCH_2S(CH_2)_2$-piperazino-R^4 R^4: $(CH_2)_2OCO(CH_2)_2COOH$	D[g)]	118~122(dch), 129~130(zml)
33	H	Et	$COCH_2S(CH_2)_2$-piperazino-Me	D	220~222(dch), 160(zml)
34	H	Et	$COCH_2S(CH_2)_2$-piperazino-$(CH_2)_2OH$	D	135~140(dch), 143~145(zml)
35	H	Et	$COCH_2S(CH_2)_2$-piperazino-$(CH_2)_2OAc$	D	133~135 (dch)
36	H	vinyl	$COCH_2S$-C_6H_4-2-OH	D	155~158
37	H	vinyl	$COCH_2S$-C_6H_4-4-OH	Lit.[15]	165~167
38	H	vinyl	$COCH_2S$-C_6H_4-2-COOH	Lit.[15]	186~188 (Na[g)])
39	H	Et	$COCH_2S$-C_6H_4-4-OH	D	191~194
40	H	vinyl	$COCH_2S$-C_6H_4-2-$O(CH_2)_2NEt_2$	E	95[a)] (ch)
41	H	vinyl	$COCH_2S$-C_6H_4-4-$O(CH_2)_2NMe_2$	E	119~123[a)] (ch)
42	H	vinyl	$COCH_2S$-C_6H_4-4-$O(CH_2)_2NEt_2$	E	88~93[a)] (ch)
43	H	vinyl	$COCH_2S$-C_6H_4-4-$O(CH_2)_3$-morpholino	E	105~110[a)] (ch)

(to be continued)

Table 1. (Continued)

No.	R^1	R^2	R^3	Method of synthesis	Mp, °C (Salt)
44	H	Et	$COCH_2S\ C_6H_4\text{-}4\text{-}O(CH_2)_2NEt_2$	E	88~92[a] (ch)
45	H	vinyl	H	Lit.[2,3,9]	192~193
46	H	Et	H	Lit.[3,9]	223~224
47	H	vinyl	$COCH_2OCO(CH_2)_5NMe_2$	F	54[a] (hfu)
48	H	vinyl	$COCH_2S(CH_2)_2CN$	D	105~107
49	H	vinyl	$CO(CH_2)_5NMe_2$	F	212~216[a] (ch)
50	$CO(CH_2)_5NMe_2$	vinyl	H	F	70[a] (ch)
51	$CO(CH_2)_5NMe_2$	vinyl	$CO(CH_2)_5NMe_2$	F	91~96[a] (ch)
52	H	vinyl	$COCH_2OCO(CH_2)_2COOH$	Lit.[15]	— (Na)
53	H	vinyl	$COCH_2OCONHR^4$ R^4: $CHCH_2CH(CH_3)_2$ COOH	Lit.[10]	— (Na)
54	H	vinyl	R^4: $CH(CH_2)_2COOH$ COOH	Lit.[10]	— (DiNa[h])
55	H	vinyl	R^4: $CHCH_3$ COOH	Lit.[10]	— (Na)
56	H	vinyl	$COCH_2S(CH_2)_2NH_2$	D	118~120[a] (hfu)
57	H	vinyl	$COCH_2S(CH_2)_2NHC(=NH)NH_2$	G	106~110[a]
58	H	vinyl	$COCH_2S(CH_2)_2NHC$ (2-imidazolyl)	G	100~105[a] (ch)
59				Lit.[3,9]	137
60				Lit.[9]	194
61	H	vinyl	$COCH_2OAc$	Lit.[9]	120~121
62	Ac	vinyl	$COCH_2OAc$	Lit.[3,9]	144~145
63	Ac	vinyl	Ac	Lit.[3,9]	199~200
64				Lit.[3,9]	181~182
65				Lit.[3,9]	106~107
66				Lit.[3,9]	191

a) Amorphous solid, melts with softening
b) Hydrogen maleate
c) Hydrochloride
d) Dihydrochloride
e) Hydrogenfumarate
f) Dihydrogenmaleate
g) Sodium salt
h) Disodium salt

ture was stirred at room temperature for 15 hours. After evaporation of most of the solvent *in vacuo*, the residue was redissolved in dichloromethane and converted to the hydrochloride by adding 15 ml of 5 N HCl in absolute ether. The solvents were again evaporated, the residue dissolved in 200 ml of a mixture of methanol and water (1:4) and the solution was extracted 4 times with ether. Neutralization of the aqueous layer with sodium hydrogen carbonate, extraction with ether (3 portions) and evaporation yielded 4.5 g (46%) of the oily base **11**. The hydrogen fumarate was prepared by dissolving 1.2 g of fumaric acid in a solution of the base **11** in dichloromethane (50 ml). The mixture was concentrated to half of its volume, diluted with ether and stirred to yield 4.42 g of a white powder which was dried at 55°C *in vacuo*. mp 80°C (softening).

Method D:

Sodium, 1.30 g (56.5 mg atom), was dissolved in 50 ml absolute ethanol, and 3.40 g (20.0 m mole) of finely pulverized diethylaminoethanethiol hydrochloride, dried over phosphorus pentoxide, were added in portions to the resulting solution under nitrogen. A solution of 10.60 g (20.0 m mole) of 14-deoxy-14-tosyloxyacetoxymutilin in 30 ml of ethylmethyl-ketone was then added dropwise while stirring.

The reaction solution was stirred at room temperature for a further 4 hours and then evaporated *in vacuo* almost to dryness. The residue was taken up in ethyl acetate and water, the organic layer washed with water until free from sodium tosylate (4 portions, check by TLC) and the solvent was removed *in vacuo*.

Purification by conversion into the hydrochloride and extraction with ether was done exactly as described in Method C for compound **11**. The yield of viscous oily base **13** was 8.9 g (90%). For the preparation of the crystalline hydrogen fumarate the base was dissolved in acetone (15 ml/g). Solid fumaric acid (1 equivalent) was dissolved while heating the mixture. After filtration the solution was set aside for crystallization. The crystals were collected by suction and dried at 40°C and subsequently at 60°C overnight. The yield was 90%, containing 1 mole acetone, mp 96~97°C. To remove the solvate acetone the salt was stirred vigorously in ethylacetate at 60°C for 6 hours. After drying overnight at 60°C and subsequently at 80°C crystals melting at 147~148°C were obtained.

The piperazine derivatives **28**~**35** readily crystallize as the dihydrochlorides, which were prepared by addition of a slight excess of 5 N HCl in absolute ether to the solution of the free base in dichloromethane. Dilution with more absolute ether yields the crystalline dihydrochlorides. The bishydrogenmaleates may be similarly prepared from the aforementioned piperazine derivatives as described for compound **6** by Method B.

From a number of bases no crystalline salt could be obtained. They were converted into their foamy hydrochlorides.

Method E:

Under nitrogen, 0.96 g (2.0 m mole) of 14-deoxy-14-(4-hydroxyphenylthio) acetoxymutilin (compound **37**) were dissolved in 20 ml of absolute methanol, 0.12 g (2.24 m mole) of sodium methoxide added, and the mixture was evaporated to dryness *in vacuo*. The residue was taken up in a mixture of 4 ml of water and 20 ml of xylene; 0.36 g (2.08 m mole) of diethylaminoethyl chloride hydrochloride and 0.42 g (3.00 m mole) of potassium carbonate were added, and the mixture was heated at reflux at a bath temperature of 150°C for 8 hours. The xylene layer was subsequently washed 5 times with water, the combined aqueous phases were extracted once with ethyl acetate, and the combined organic phases concentrated by evaporation *in vacuo*. The residue was dissolved in 10 ml of dichloromethane, converted into the hydrochloride and purified by extraction with ether as described in Method C. The yield was 0.71 g (58%) of a foamy white salt (compound **42** hydrochloride), homogeneous by TLC in chloroform-methanol (1:2). mp 90°C (softening).

Method F:

Mutilin (compound **45**) (3.20 g, 10.0 m mole) and 6-dimethylaminohexanoylchloride hydrochloride (3.50 g, 16.0 m mole) were dissolved in 30 ml of absolute dimethylformamide and heated for 16 hours at 110°C. The solvent was then removed *in vacuo*. The residue was taken up in 50 ml of dichloromethane and the basic compounds were separated by conversion into the hydrochloride and extraction with ether (see Method C). The yield was 4.2 g of crude bases. Separation of the isomeric bases was achieved by preparative TLC in chloroform - methanol (7:1) on silica gel plates (2 mm layer thickness), prepared by spraying three times with 0.1 N aqueous $AgNO_3$ and each time dried before use. From the faster moving zone compound **50** (11-O-acyl derivative) was isolated. The lower moving band yielded compound **49** (14-O-acyl derivative) and a band near the start contained compound **51** (11,14-di-O-acyl derivative). The free bases **49**, **50** and **51** were converted into the foamy hydrochlorides with HCl in ether. They decompose on heating.

NMR: $\delta^{CDCl_3}_{ppm}$ **49**: 3.44 (d, J=7 Hz, H-11); 5.78 (d, J=8 Hz, H-14).
50: 4.31 (d, J=8 Hz, H-14); 4.89 (d, J=7 Hz, H-11).
51: 4.90 (d, J=7 Hz, H-11); 5.65 (d, J=7 Hz, H-14).

For comparison compound **1** (pleuromutilin):
3.40 (d, J=6.5 Hz, H-11), 5.84 (d, J=8.5 Hz, H-14).

Compound **45** (mutilin):
3.40 (d, J=6.5 Hz, H-11); 4.34 (d, J=7.5 Hz, H-14).

Compound **62**: 4.93 (d, J=6.5 Hz, H-11); 5.76 (d, J=8.5 Hz, H-14).

6-Aminohexanoic acid, 13.1 g (0.1 mole), was heated for 6 hours at reflux together with 18.9 ml (0.5 mole) formic acid and 16.6 ml (0.22 mole) 37% aqueous formaldehyde. The vigorous evolution of

carbon dioxide ceased after about one hour. The water and excess formic acid were evaporated *in vacuo* at 80°C. The residue was dissolved in 5 N HCl and evaporated to dryness three times. To this hydrochloride 8 ml of thionylchloride were added drop by drop while cooling. After 2 hours at room temperature the excess thionylchloride was removed under reduced pressure. The resulting solid hydrochloride of 6-dimethylaminohexanoyl chloride was used for the above preparation.

Method G:

Three grams (6.46 m mole) of 14-deoxy-14-[(2-aminoethyl)-thioacetoxy]-mutilin(compound **56**) were dissolved in 50 ml of absolute dimethylformamide and a solution of 0.86 g (6.2 m equivalents) of 5-methylisothiouronium sulfate in 5 ml of water was added. The mixture was heated for three hours at 100°C. After removal of the solvents *in vacuo* the residue was dissolved in methanol and 40 ml of 5 N HCl in ether were added while cooling in ice. The solvents and excess hydrogen chloride were again evaporated. The residue was dissolved in 50 ml of methanol and converted into the free base on a column filled with an anion-exchange resin (Dowex 1×4, 50~100 mesh, OH^- form). Elution with methanol, evaporation, stirring with absolute ether and drying *in vacuo* yielded 2.80 g (95%) of compound **57** as a white powder.

By the same procedure compound **58** was synthesized using 2-methylthio-imidazoline hydroiodide and compound **56**.

Method H:

To a solution of 1.2 g (5.6 m mole) of 6-dimethylaminohexanoylchloride hydrochloride (Method F) in 10 ml of absolute dimethylformamide were added 1.60 g (4.25 m mole) of pleuromutilin. The mixture was heated for 30 minutes at 110°C, the solvent evaporated *in vacuo* and the product purified by conversion into the hydrochloride, ether extraction and final conversion into the hydrogen fumarate as described in Method C. The yield of hydrogen fumarate was 1.15 g (43%).

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