STUDIES ON PLEUROMUTILIN AND SOME OF ITS DERIVATIVES

K. RIEDL

Research and Development Department, Biochemie Ges. m.b.H., Kundl/Austria

(Received for publication August 11, 1975)

A number of derivatives of pleuromutilin (**I**) and of its degradation product, mutilin (**II**), was prepared. The new monotosylation product of pleuromutilin (**IIIc**) served as the key substance for modification of the glycolic acid side chain. From the pleuromutilin monosuccinate (**IIIk**) water-soluble salts were obtained, among them the crystallized diethylaminoethanol salt that was investigated more closely. Some of the pleuromutilin derivatives showed antimicrobial activity.

In 1963 a white crystalline substance showing activity against certain microorganisms was isolated at Biochemie Ges. m.b.H. from cultures of a basidiomycete.[1] Identity of that substance with pleuromutilin[2] was established. This antibiotic had been initially investigated by ANCHEL,[3] but ARIGONI[4] was the first to elucidate the complete structure. The structure was confirmed by BIRCH and co-workers.[5,6] Interesting details concerning chemistry, stereochemistry and biochemistry can be found in dissertations of members of ARIGONI's group.[7~9]

In the years 1963~1966 we were working on the chemical modification of pleuromutilin. A number of reactions were tried, resulting in a variety of new compounds.

Pleuromutilin (**I**) is the glycolic acid ester of mutilin (**II**), a novel type of diterpene (Table 1). Because of its structure, difficulties in modifying the molecule can be expected: the number of functional groups is small and only mild and selective methods can avoid undesired modification of the molecule.

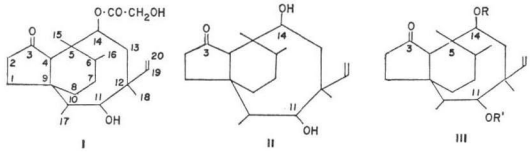
By oxidation of pleuromutilin with activated manganese dioxide we found a new compound that showed no antibiotic activity. It was soluble in aqueous bicarbonate, did not migrate in our TLC systems (see preparative part) and was concluded to be a carboxylic acid from its IR spectrum.

Based on its CH-value, equivalent weight and a degradation experiment with alkali (leading to mutilin and oxalic acid) we were able to elucidate the structure of this compound (**IIIa**). This oxidative reaction is remarkable because oxidations hitherto performed had yielded only 11-deoxy-11-oxo-pleuromutilin[10] and 11-deoxy-11-oxo-mutilin monooxalate[6] respectively. By methylation of **IIIa** with diazomethane the biologically active methylester **IIIb** could be obtained.

For modifying the side chain, we started from the consideration that the activation of this part of the molecule *via* sulfonic acid esters would give the opportunity for numerous exchange reactions. Monotosylation was easily performed, yielding 14-deoxy-14-*p*-tosyloxyacetoxy-mutilin (**IIIc**) a product that was useful as starting material for synthesis of derivatives[11,12] with antimicrobial activity. The tosyloxy-group in this compound was exchanged smoothly for Cl(**IIId**), Br, I, SCN (**IIIe**), SR (**IIIf**) and N_3 (**IIIg**). Transformation of **IIIc** to **IIIf** led to compounds with enhanced antimicrobial activity.

In some cases these primary reaction products could be further transformed: by catalytic

Table 1. Structures of pleuromutilin (**I**), mutilin (**II**) and derivatives produced thereof (**III**)



I II III

Substance	R	R′
IIIa	$-CO\cdot COOH$	H
IIIb	$-CO\cdot COOCH_3$	H
IIIc	$-CO\cdot CH_2\cdot O\cdot SO_2\cdot C_6H_4\cdot CH_3$	H
IIId	$-CO\cdot CH_2Cl$	H
IIIe	$-CO\cdot CH_2\cdot SCN$	H
IIIf	$-CO\cdot CH_2\cdot S\cdot R''$	H
IIIg	$-CO\cdot CH_2\cdot N_3$	H
IIIh	$-CO\cdot CH_2\cdot NH_2$	H*
IIIi	$-CO\cdot CH_3\cdot$	H
IIIj	$-CO\cdot CH_2\cdot OH$	$-COCH_3$
IIIk	$-CO\cdot CH_2\cdot O\cdot CO(CH_2)_2\cdot COOH$	H

* dihydro-compound

Table 2. Activities of pleuromutilin derivatives against some strains of *Staphylococcus aureus*.

Derivative	MIC values (mcg/ml)		
	I	II	III
IIIc	6.2	502	350
14-deoxy-14-bromo-acetoxymutilin	0.5	252	200
14-deoxy-14-iodo-acetoxymutilin	0.47	4.3	75
IIIe	0.46	49	200
IIId	0.3	250	200
IIIg	0.6	254	200
IIIi	0.2	250	200
IIIh	26	69	90
IIIk	1.8	27	41
IIIf	0.03	250	200

The strains of *Staphylococcus aureus* are grouped according to their sensitivity to penicillin G.
I = 7 sensitive strains (MIC 0.03 mcg/ml)
II = 8 moderately sensitive strains (MIC 1. 6 mcg /ml)
III = 10 highly resistant strains (MIC 84 mcg/ml)

All strains were from the Biochemie Ges. m.b.H. stock culture collection. The tests were carried out by a serial tube dilution method (Difco antibiotic medium 3).

Table 3. Thin-layer chromatography
TLC was performed on silica gel plates (Merck). Solvent systems used: Diisopropyl ether and toluene-ethyl acetate (1:1). Color development: The plates were sprayed with a mixture of 0.1 g of ferric chloride, 1 ml of glacial acetic acid and 99 ml of concentrated sulfuric acid and heated to 110°C. Yellowish-brown spots resulted except for the 11-oxo-compounds which gave spots violet. From a number of chromatograms, the following average values of Rf resulted:

Rf	Diisopropyl ether	Toluene-ethyl acetate (1:1)
0.00	h	—
0.06~0.08	c. P, H(f)	h
0.11~0.12	e, b	—
0.13~0.14	P(f), d	—
0.15	g, X(f), M, i	—
0.23~0.24	j, C	—
0.28~0.29	—	P
0.33~0.34	PD	H(f)
0.36	M 11	—
0.39~0.40	—	M, c, e, b,
0.43~0.45	MD	i, d, g, P(f)
0.48	—	j, X(f)
0.57	—	M 11
0.59	—	PD
0.63~0.65	—	MD

Small letters mark the corresponding compounds of type III. Other abbreviations: P=pleuromutilin, M=mutilin, X(f)=ethylxanthogenate (f), P(f)=phenylmercaptoacetyl (f), H(f)=*p*-hydroxyphenylmercaptoacetyl (f), C=11-oxo-c, M11=mutilin-11-acetate, PD=pleuro-mutilindiacetate, MD=mutilindiacetate.

reduction of the azido compound (**IIIg**) we obtained the aminodihydro compound (**IIIh**)—apparently the first pleuromutilin derivative with a basic group—and prepared also its salts. By reaction of **IIIe** or the bromo compound (**III**, R=$BrCH_2CO$, R′=H) with aluminum amalgam in ethanol we obtained a product that proved to be a monoacetate of mutilin (**IIIi**). The same compound could be prepared microbiologically from mutilin.[1] Likewise it resulted from partial alkaline saponification of mutilin diacetate. The preparation of an

isomeric product by careful acetylation of mutilin with acetic anhydride is described in the literature.[13]

By cleavage of diacetylpleuromutilin with alcoholic hydrochloric acid we obtained 11-deoxy-11-acetoxypleuromutilin (**IIIj**), isomer to pleuromutilin monoacetate described in the work of BIRCH *et al.*[8]

Oxidation of the 14-deoxy-14-p-tosyloxylacetoxymutilin (**IIIc**) with chromium trioxide led to the corresponding 11-ketone.

A number of the derivatives with intact ester functions and 11-hydroxyl group showed antibiotic activity. However, since all these compound were only slightly soluble in water, water-soluble derivatives were also sought. The succinic acid semi-ester of pleuromutilin (**IIIk**) was prepared from the antibiotic by reaction with succinic anhydride. This ester gave a crystalline diethylaminoethanol salt that is readily soluble in water. Aqueous solutions were fairly stable at room temperature and showed activity against some microorganisms.

Experimental

All melting points were taken with a heating microscope (Kofler). IR spectra were obtained with the Perkin Elmer 237 spectrophotometer, optical rotations were recorded on a Perkin Elmer 141 polarimeter (chloroform, dm-tube).

Reaction of pleuromutilin with activated manganese dioxide

(**a**) A suspension of 1.8 g of pleuromutilin in 50 ml of hexane was boiled with 15 g activated manganese dioxide (containing some alkali). Soon a semisolid mass formed that made it difficult to stir up the manganese dioxide. After four hours solid was removed by suction, washed with acetone and extracted with boiling ethanol. Hexane and acetone solutions were discarded. After evaporation of the ethanol extracts a white residue was obtained which was suspended in acetone and filtered off. Yield 0.33 g. This substance was dissolved in water and acidified. A crystalline compound was isolated, that melted at 205~208°C (decomp.) after recrystallization from chloroform - hexane.

(**b**) A mixture of 3.4 g of pleuromutilin, 30 g of activated manganese dioxide and 2 g of anhydrous potassium carbonate was heated in 50 ml of acetone to gentle boiling for 4~5 hours. The manganese dioxide was removed by suction and washed with acetone. The solvent was evaporated leaving an oily residue of 0.7 g.

The manganese dioxide was brought to boiling with 250~300 ml of absolute ethanol and removed by suction. Clarifying of the turbid filtrate by Celatom was necessary. The evaporated extract yielded a residue that was twice suspended in benzene and re-evaporated. By that procedure better solidification of the product was achieved. Addition of acetone and stirring with a glass rod brought about precipitation of the potassium salt. This solid was removed and washed with acetone. The yield of the product was 1.5 g. It was dissolved in 50 ml of water, filtered through Celatom and the solution acidified with 6N hydrochloric acid. Thus a crystallined substance (**IIIa**) was obtained that could be recrystallized from benzene, chloroform-hexane or ethylacetate.

Anal. Calcd. for $C_{22}H_{32}O_6$: C 67.32 H 8.21
Found: C 66.90 H 8.27

Equivalent weight, calcd. for one carboxyl group: 392.49. Found: 397.00

Saponification of **IIIa** with alkali

A solution of 0.2 g of the free acid in 10 ml of ethanol was refluxed with 1.5 ml of N sodium hydroxide for two hours on the water bath. After evaporation of the solvent and addition of a small volume of water to the residue, the solid was filtered off and recrystallized from a small amount of ethyl acetate. The mixed m.p., TLC and IR spectrum indicated that

this cleavage product was mutilin. In the aqueous solution (filtrate) oxalic acid was found.

Esterification of **IIIa** with diazomethane

To a solution of 0.2 g of **IIIa** in 5 ml of peroxide-free tetrahydrofuran was added diazomethane in ether until the yellow color persisted. The oil obtained after evaporation of the ether crystallized after addition of hexane. Recrystallized from ethyl acetate the substance melted at 128~131°C. IR spectrum (CCl_4): 3640, 3560, 1770, 1740, 1205, 1170, 1155, 1115, 1018, 933, 918 cm^{-1}. $[\alpha]_D^{20}$ +23.6° (*c* 1).

Anal. Calcd. for $C_{23}H_{34}O_6$: C 67.95, H 8.43
Found: C 67.60, H 8.50

14-Deoxy-14-*p*-tosyloxyacetoxymutilin (**IIIc**)

A solution of 95 g of pleuromutilin in 350 ml of pyridine was cooled to −15°C for 20 minutes. Then *p*-toluene sulfonylchloride (65 g) was added in one portion and shaken until completely dissolved. The solution was kept for two hours at −15°C and repeatedly shaken. After an additional hour at 0°C the solution was added to 300~400 ml of ice-water and extracted with 350 ml of chloroform. The cold organic phase was washed once with ice-water, three times with 3 N sulfuric acid, then with water and finally with saturated sodium bicarbonate solution. The organic phase was dried with sodium sulfate, evaporated and the residue triturated with hexane. Crystallization occured on standing overnight. The product was contaminated with starting material that could be removed either by chromatography (Al_2O_3) or by recrystallization from isopropanol. After recrystallization of the crude material from 350 ml of isopropanol we obtained 80 g of **IIIc**, m.p. 146~148°C. (60 % of theory). For analysis it was recrystallized twice from isopropanol and once from acetone-hexane. M.p. 147.5~149.5°C.

Anal. Calcd. for $C_{23}H_{40}O_7S$: C 65.38, H 7.56
Found: C 65.02, H 7.70

IR spectrum (KBr): 3520, 3450, 3080, 1740~1730, 1630, 1598, 1190, 1180, 838, 820, 665 cm^{-1}
($CHCl_3$): 3560, 1755, 1735, 1730, 1598, 1175, 1015 cm^{-1}
$[\alpha]_D^{20}$ +18.7° (*c* 1).

Oxidation of **IIIc** with chromium trioxide

A solution of 21.2 g of 14-deoxy-14-*p*-tosyloxyacetoxymutilin in 250 ml of acetic acid was treated at 10~15°C with portions of a solution of 8 g of chromium trioxide in 50 ml of glacial acetic acid with stirring until the color of the reagent persisted. Stirring was continued for an hour at ice-water temperature. The solution was then poured into ice-water and the mixture extracted with chloroform. The chloroform extract was washed with three portions of aqueous sodium bicarbonate and evaporated. An oily residue was obtained that crystallized by treating with isopropanol. Yield: 14.8 g. M.p. 115~118°C. (70 % of theory). For preparation of a sample for anlysis, a product twice recrystallized from benzene was chromatographed on neutral aluminum oxide (Woelm) and finally recrystallized from acetone-hexane. The thoroughly dried, colorless product had a m.p. 116~118°C.

Anal. Calcd. for $C_{29}H_{38}O_7S$: C 65.64, H 7.22
Found: C 65.26, H 7.34

IR spectrum (CCl_4): 1760, 1740, 1700, 1190, 1180 cm^{-1}

14-Deoxy-14-chloroacetoxymutilin (**IIId**)

To a solution of 10.5 g of 14-deoxy-14-*p*-tosyloxyacetoxymutilin in 50 ml of acetone a suspension of 1 g of lithium chloride in 70 ml of acetone was added and kept at 65°C (to achieve gentle boiling and avoid bumping) for 6 hours. After completion of the reaction the lithium salt was removed by suction, the solution evaporated and the residue dissolved in chloroform. After washing of the organic phase with water and drying with sodium sulfate the solvent was removed and the oily residue crystallized by addition of hexane. The crude product showed a single spot on TLC. Yield: 6.0 g(76 % of theory). For analysis it was

recrystallized from isopropanol and finally from acetone-hexane. M.p. 123~124°C.

Anal. Calcd. for $C_{22}H_{33}ClO_4$: C 66.56, H 8.38
Found: C 66.44, H 8.48

IR spectrum: Cl at 788 cm^{-1}.

14-Deoxy-14-iodoacetoxymutilin

This compound was synthesized from 52 g of **IIIc**, 17 g of sodium iodide and 350 ml of acetone. Yield of crude product: 42.6 g (87 % of theory). The substance could be recrystallized from isopropanol with loss. M.p. 118~120°C.

Anal. Calcd. for $C_{22}H_{33}IO_4$: C 54.10, H 6.80
Found: C 53.85, H 6.90

14-Deoxy-14-bromoacetoxymutilin

Similarly 43 g of crude bromo compound was obtained from 53 g of **IIIc**, 12 g of sodium bromide, 400 ml of acetone and 80 ml of water. On TLC, impurities were found. After repeated recrystallizations from isopropanol the substance melted at 118~121°C.

Anal. Calcd. for $C_{22}H_{33}BrO_4$: C 59.85, H 7.53
Found: C 59.40, H 7.65

14-Deoxy-14-thiocyanatoacetoxymutilin (**IIIe**)

A mixture of 5.3 g of **IIIc**, 1.1 g of potassium thiocyanate and 70 ml of methyl ethyl ketone was heated half an hour on the water bath. The yield of precipitated potassium tosylate was practically quantitative. After evaporation of the solvent the residue was purified by distribution between chloroform and water. The dried chloroform phase was concentrated to an oil, that recrystallized on addition of hexane. Yield: 3.8 g (90.6 % of theory). The product was homogeneous according to TLC and had a m.p. 134~136°C after recrystallization from isopropanol. For analysis it was twice recrystallized from the same solvent and dried at 60°C *in vacuo*.

Anal. Calcd. for $C_{23}H_{33}NO_4S$: C 65.84, H 7.93
Found: C 65.92, H 8.12

The IR spectrum showed the isothiocyanate band at 2155 cm^{-1}.

14-Deoxy-14-phenylmercaptoacetoxymutilin (**IIIf**, $R=C_6H_5SCH_2CO$, $R'=H$)

To a solution of 0.46 g of sodium in 50 ml of methanol thiophenol (2.2 ml) was added and the mixture cooled to ice-water temperature. **IIIc** (10.6 g), dissolved in 30 ml of acetone, was added by means of a dropping funnel. After standing for an hour at room temperature, the mixture was heated on the boiling water bath for 15 minutes and taken to dryness. The residue was treated with water and chloroform and the organic phase worked up as usual. Yield: 7.7 g, m.p. 120~123°C (81.8 % of theory). The compound crystallized from acetone-hexane in large needles.

Anal. Calcd. for $C_{28}H_{38}O_4S$: C 71.46, H 8.14
Found: C 71.26, H 8.25

IR spectrum (Nujol): 3520, 3100~3000, 1720, 1710, 1640, 1580, 1275, 742 cm^{-1}.

To verify the structure of the substituent 2.3 g of the above product, 1.5 g of potassium hydroxide, 6 ml of water and 25 ml of ethanol were heated for 2 hours on the water bath. After evaporation of the alcohol, the residue was diluted with 75 ml of water, filtered and extracted with chloro-form and subsequently with hexane. The aqueous phase was then concentrated, acidified and a quantity of ammonium sulfate added. The crystallized substance was removed by suction, washed with a minimum amount of water and dried. The solid melted at 60~63°C and its IR spectrum and behavior on TLC indicated the identity to phenylmercaptoacetic acid.

14-Deoxy-14-*p*-hydroxyphenylmercaptoacetoxymutilin (**IIIf**, $R=HOC_6H_4SCH_2CO$, $R'=H$)

To a mixture of 10.6 g of **IIIc**, 3.15 g of thiohydroquinone and 30 ml of acetone a solution of 0.5 g of sodium in 15 ml of absolute ethanol was added rapidly (cooling, N_2-atmosphere).

After shaking for 15 minutes, the reaction mixture was filtered and the filtrate heated for 20 minutes on the water bath. Acidification with acetic acid and evaporation gave a residue which was distributed between water and ethyl acetate. Evaporation of the dried organic phase yielded an oil that solidified on standing. Recrystallization from chloroform-hexane gave 8 g of a product which showed a single spot in several solvent systems on TLC, but did not melt sharply (82.2 % of theory).

Anal. Calcd. for $C_{28}H_{38}O_5S$: C 69.10, H 7.86
Found: C 68.85, H 8.10

IR spectrum (KBr): 1735~1730, 1700~1690, 1600, 1580, 835 cm^{-1}.

Similarly 14-deoxy-14-(2′, 5′-dihydroxyphenylmercaptoacetoxy) mutilin (**IIIf**, R=2, 5-dihydroxyphenylmercaptoacetyl, R′=H) and 14-Deoxy-14-(2′-carboxyphenylmercaptoacetoxy) mutilin (**IIIf**, R=2-carboxyphenylmercaptoacetyl, R′=H) were prepared.

Anal. Calcd. for $C_{28}H_{38}O_6S$: C 66.90, H 7.61
Found: C 66.38, H 7.75
Anal. Calcd. for $C_{29}H_{38}O_6S$: C 67.67, H 7.44
Found: C 67.50, H 7.58

14-Deoxy-14-β-hydroxyethylmercaptoacetoxymutilin (**IIIf**, R=$HOCH_2CH_2SCH_2CO$, R′=H).

This compound was prepared from **IIIc** and β-hydroxyethylmercaptan. The product was a viscous oil which did not crystallize after prolonged standing. On TLC one spot appeared.

Anal. Calcd. for $C_{24}H_{38}O_5S$: S 7.3
found: S 7.6

Reaction of **IIIc** with potassium xanthogenate (**IIIf**, R=$C_2H_5OCSSCH_2CO$, R′=H)

To 15.9 g of **IIIc** in acetone a warm solution of 4.8 g of potassium xanthogenate in acetone was added. Immediately potassium tosylate precipitated. The precipitate was centrifuged and washed with acetone. The combined acetone solutions were evaporated yielding a yellow oil (14.7 g) that crystallized by triturating with isopropanol. For analysis the compound was recrystallized twice from isopropanol.

Anal. Calcd. for $C_{25}H_{38}O_5S_2$: C 62.20, H 7.94
Found: C 61.96, H 8.04

14-Deoxy-14-azidoacetoxymutilin (**IIIg**)

A solution of 26.6 g **IIIc** in 250 ml of acetone was mixed with a solution of 3.5 g of sodium azide in 32 ml of water and the mixture heated for 3 hours on a water bath. After evaporation to dryness, the residue was shaken with chloroform and water, and the washed organic layer evaporated after drying with sodium sulfate. The residue gave 19.3 g of crude material. The substance was twice recrystallized from isopropanol and once from acetone. M.p. 135~140°C (decomp).

Anal. Calcd. for $C_{22}H_{33}O_4N_3$: C 65.48, H 8.24
Found: C 65.45, H 8.27

IR spectrum (KBr): 2110 (N_3), 1735, 1720~1710 cm^{-1}.

Hydrogenation of 14-deoxy-14-azidoacetoxymutilin

Four grams of **IIIg** were hydrogenated in 50 ml of glacial acetic acid with 1.5 g of Pd on carbon (10 %). After shaking for several hours and repeated flushing with air, the catalyst was filtered off and the filtrate evaporated at low temperature as completely as possible. Water was added to the residue and the insoluble portion removed by filtration. By addition of sodium bicarbonate to the filtrate, a substance was precipitated that melted after recrystallization from a small amount of isopropanol at 178~182°C (decomp.) (**IIIh**). The base can be precipitated as hydrochloride with hydrogen chloride in ether.

Anal. Calcd. for $C_{22}H_{36}ClNO_4$: C 63.82, H 8.76
Found: C 63.75, H 8.81

IR spectrum (hydrochloride, KBr): 2660, 2460, 2020, 1735~1710, 1630~1590 cm^{-1}.

Reaction of 14-deoxy-14-thiocyanatoacetoxymutilin with aluminum amalgam

A solution of 15 g **IIIe** in 300 ml of absolute ethanol was boiled with 10 g of amalgamated coarse aluminum. After 3 hours the pasty reaction product was concentrated *in vacuo* and the residue treated with chloroform and water. Then 200 ml of ice-cold 6N hydrochloric acid were added the chloroform-layer separated, washed with water and dried with sodium sulfate. The solvent was removed by evaporation and the residue crystallized by addition of hexane. Yield: 10 g (**IIIi**). For analysis this product was purified by recrystallization from isopropanol or a small amound of acetone. M.p. 185~186°C (with sublimation).

Anal. Calcd. for $C_{22}H_{34}O_4$: C 72.89, H 9.45
Found: C 72.62, H 9.55

IR spectrum (KBr): 3500, 3080, 1700, 1633, 1275, 1025, 915 cm^{-1}.
(CCl_4): 3570, 3080, 1740, 1630, 1245, 1018 cm^{-1}.

Oxidation of **IIIi** with chromium trioxide

A mixture of 150 mg of **IIIi**, 12 ml of 85 % acetic acid and 200 mg of chromium trioxide was kept for 5 hours at room temperature. Then the excess chromium trioxide was reduced with methanol and the solution, after concentration was poured into ice-water. Ether extraction yielded a substance with m.p. 125~126°C (from ethyl acetate).

IR spectrum (CCl_4): 3080, 1740~1720, 1700, 1630, 1245, 1218 cm^{-1}.

Anal. Calcd. for $C_{22}H_{32}O_4$: C 73.30, H 8.94
Found: C 73.04, H 8.88

Reduction of **IIIi** with lithium aluminum hydride

A solution of 150 mg of **IIIi** in 5 ml of tetrahydrofuran was added dropwise to a suspension of 0.6 g of lithium aluminum hydride in 50 ml of the same solvent. Subsequently the mixture was heated for 3 hours on a water bath. After decomposition of excess lithium aluminum hydride with ethyl acetate and addition of water, 173 mg of an oil could be obtained by evaporation of the organic solvent. Recrystallized from benzene, the substance melted at 181~182°C. Its identity to 3-deoxo-3-hydroxymutilin[14] was proved by IR spectrum and TLC.

Partial saponification of mutilin-diacetate with alkali

A mixture of 0.4 g of mutilin-diacetate, 0.1 g of KOH and 10 ml of ethanol was refluxed on a steam bath for 2 hours. After evaporation of the ethanol, water was added to the residue, the product extracted with ether and recrystallized from a small amount of ethanol. TLC (silicagel 60/Merck, ethylacetate - toluene 1:1) showed spots at R_f 0.67 (starting material), 0.49 (main spot) and 0.45 (trace of mutilin). After recrystallization of the main product (obtained by preparative TLC) from ethyl acetate, its identity to **IIIi** was proved by IR.

Cleavage of pleuromutilin-diacetate with ethanolic hydrochloric acid

To 2.3 g of pleuromutilin-diacetate[3] (0.005 moles, m.p. 142~144°C), 40 ml of 0.12N ethanolic hydrochloric acid were added and the mixture boiled on a water bath for 3 hours. After evaporation of the clear solution, a resin was obtained that turned powdery by triturating with hexane. Yield: 2.0 g. Recrystallization from ethanol gave m.p. 160~162°C.

IR spectrum (KBr): 3420, 1770, 1730, 1240, 1110 cm^{-1}.

Anal. Calcd. for $C_{24}H_{36}O_6$: C 68.54, H 6.62
Found: C 68.60, H 8.80

The reduction of this compound with lithium aluminum hydride gave 3-deoxo-3-hydroxymutilin[14] (TLC, IR). Acetylation (0.2 g of the substance in 5 ml of acetic anhydride - pyridine (1:4) at room temperature for 4 days) converted the compound to pleuromultilin diacetate (mixed m.p. and IR). The cleavage product by ethanolic hydrochloric acid was therefore concluded to be **IIIj**, as the isomer 14-deoxy-14-acetoxyacetoxymultilin[6] is a known substance and differs in m.p. and IR-spectrum.

Preparation of the diethylaminoethanol salt of mono-pleuromutilin-succinate (**IIIk**)

A mixture of 380 g of pleuromutilin (recrystallized from ethyl acetate), 100 g of succinic

anhydride, 140 ml of triethylamine and 2 liters of toluene was shaken for 8 hours. Soon a crystalline reaction product was observed. After standing overnight the content of the flask was transferred to a separatory funnel and 2.5 liters of water and 110 g of sodium bicarbonate were added. By shaking the solid dissolved. The toluene phase was discarded and the bicarbonate phase washed once with ether. Then the aqueous phase was acidified with phosphoric acid to pH 2 and extracted with ether, the ether extract washed with water and dried over sodium sulfate. After filtration a volume of 3,740 ml resulted. By addition of 116 ml of diethylaminoethanol in 200 ml of ether, a viscous oil separated that crystallized on scratching. The solid was collected on a Büchner funnel and dried at 40°C *in vacuo*. Yield: 490~500 g. For analysis the salt was recrystallized from acetone.

Anal. Calcd. for $C_{32}H_{53}NO_9$: C 64.51, H 8.96
Found: C 64.50, H 9.06

This salt melted at 122~125°C with decomposition and was readily soluble in water (about 30 %). The aqueous solution had a pH of 6.1. In concentrated aqueous solutions of the salt only traces of pleuromutilin were formed by hydrolysis after standing for 24 hours at room temperature (detected by TLC).

Acknowledgements

The author wishes to thank Mr. U. WOWORSKY for helpful assistance, Mr. O. TOTSCHING for elementary analyses and Dr E. SCHMID for microbiological tests.

References

1) KNAUSEDER, F. & E. BRANDL: Pleuromutilins. Fermentation, structure and biosynthesis. J. Antibiotics 29: 125~131, 1976
2) KAVANAGH, F.; A. HERVEY & W. J. ROBBINS: Antibiotic substances from Basidiomycetes. VIII. *Pleurotus mutilus* and *Pleurotus passeckerianus*. Proc. Natl. Acad. Sci. 37: 570~574, 1951
3) ANCHEL, M.: Chemical studies with pleuromutilin. J. Biol. Chem. 199: 133~140, 1952
4) ARIGONI, D.: Structure of a new type of terpene. Gazz. Chim. Ital. 92: 884~901, 1962
5) BIRCH, A. J.; D. W. CAMERON, C. W. HOLZAPFEL & R. W. RICHARDS: Diterpenoid nature of pleuromutilin. Chem. Ind. (London) 1963: 374~375, 1963
6) BIRCH, A. J.; C. W. HOLZAPFEL & R. W. RICHARDS: The structure and some aspects of the biosynthesis of pleuromutilin. Tetrahedron 1966, Suppl. 8, Part II, pp. 358~387, 1966
7) NAEGELI, P.: On pleuromutilin. Dissertation Nr. 3206, ETH Zürich, 1961
8) BUZZOLINI, M.; On biogenesis of pleuromutilin and the lagopodins. Dissertation Nr. 3797, ETH (Zürich). 1966
9) BONAVIA, G.: Pleuromutilin, stereochemistry and detailled biosynthesis. Dissertation Nr. 4189, ETH (Zürich). 1968
10) NAEGELI, P.: On pleuromutilin. Dissertation Nr. 3206, p. 63, 1961
11) Biochemie G.m.b.H.: Procedure for preparation of novel derivatives of pleuromutilin. Austrian Patent 301 753, 1969
12) Biochemie G.m.b.H.: 14-Deoxy-14-*p*-tosyloxyacetoxymutilin. Austrian Patent 303 962, 1969
13) NAEGELI, P.: On pleuromutilin. Dissertation Nr. 3206, p. 60, 1961
14) NAEGELI, P.: On pleuromutilin. Dissertation Nr. 3206, p. 62, 1961